REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
27-01-2016	Final Report	1-Sep-2012 - 31-May-2013

4. TITLE AND SUBTITLE
Final Report: Peptide nanotube reinforced polymers: A system for tunable, composite materials

5a. CONTRACT NUMBER
W911NF-12-1-0229

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHORS
Neel Joshi, Daniel Rubin, Rajiv Desai

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
Harvard Medical School
25 Shattuck Street

Boston, MA 02115 -6027

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
62177-LS-II.3

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
D,L-cyclic peptides (DLCPs) are peptides composed of alternating D and L amino acids that self-assemble into nanotubular stacks and microcrystalline aggregates. The goal of this proposal was to determine the suitability of these self-assembled structures for the mechanical reinforcement of polymeric materials used in the fabrication of implantable medical devices. Our results show that the high aspect ratio microcrystalline aggregates (a.k.a. "microneedles") that come from the assembly process are capable of increasing the average stiffness of

15. SUBJECT TERMS
nanotube, peptide self-assembly, polymer reinforcement

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Neel Joshi
UU	UU	UU	UU		19b. TELEPHONE NUMBER 617-432-7730

Standard Form 298 (Rev 8/98)
Prescribed by ANSI Std. Z39.18

Report Title

Final Report: Peptide nanotube reinforced polymers: A system for tunable, composite materials

ABSTRACT

D,L-cyclic peptides (DLCPs) are peptides composed of alternating D and L amino acids that self-assemble into nanotubular stacks and microcrystalline aggregates. The goal of this proposal was to determine the suitability of these self-assembled structures for the mechanical reinforcement of polymeric materials used in the fabrication of implantable medical devices. Our results show that the high aspect ratio microcrystalline aggregates (a.k.a. "microneedles") that come from the assembly process are capable of increasing the average stiffness of biodegradable polymers like poly-D,L-lactic acid (PDLLA), a common polymer used in resorbable load bearing implants. Preliminary experiments also demonstrated that, for a particular sequence of DLCP (cyclo-[Gln-Leu]4) the assembled microneedles do not exhibit any cytotoxicity toward sheep fibroblasts. Finally, nanomechanical characterization of the microneedles revealed that they were among the stiffest known proteinaceous substances in existence, suggesting their utility as mechanical reinforcers. However, significant challenges remain in rationally controlling the size and surface chemistry features of DLCP-derived microneedles, limiting the ease of their incorporation into biomedical implant devices.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
11/30/2015	2.00	Shahrouz Amini, Daniel J. Rubin, Feng Zhou, Haibin Su, Ali Miserez, Neel S. Joshi. Structural, Nanomechanical, and Computational Characterization of, ACS Nano, (03 2015): 3360. doi: 10.1021/acsnano.5b00672
11/30/2015	1.00	Daniel J. Rubin, Hadi T. Nia, Thierry Desire, Peter Q. Nguyen, Michael Gevelber, Christine Ortiz, Neel S. Joshi. Mechanical Reinforcement of Polymeric Fibers through Peptide Nanotube Incorporation, Biomacromolecules, (10 2013): 3370. doi: 10.1021/bm4008293

TOTAL: 2

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

ACS National Meeting New Orleans (2013)
MIT Synthetic Biology Group
MRS National Meeting (Boston 2013)

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

D, L-cyclic Peptide Nanotubes as Reinforcing Agents

Patents Awarded

D, L-cyclic Peptide Nanotubes as Reinforcing Agents

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Daniel Rubin	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Neel Joshi	0.08	
FTE Equivalent:	**0.08**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Brian Borsiquot	0.00	Chemistry
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 1.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 1.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 1.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 1.00

Names of Personnel receiving masters degrees

NAME	
Total Number:	

Names of personnel receiving PHDs

NAME	
Daniel Rubin	
Total Number:	**1**

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

5 D, L-cyclic peptide nanotube reinforcing agents

Patent Filed in US? (5d-1) Y

Patent Filed in Foreign Countries? (5d-2) N

Was the assignment forwarded to the contracting officer? (5e) N

Foreign Countries of application (5g-2):

5a: Daniel Rubin

5f-1a: Harvard University

5f-c: 29 Oxford St.

Cambridge MA 02143

5a: Neel Joshi

5f-1a: Harvard University

5f-c: 29 Oxford St.

Cambridge MA 02138

Scientific Progress

Final Report

1) Foreword

This is the final report for an STIR grant awarded in September 2012 and ended in May 2013. The award provided important seed funding for a project that formed the basis for one PhD thesis in my lab and helped to forge two important collaborations. Below is provided a summary of the project during and after the award period. More technical details can be found in the two publications referenced in this report.

2) Statement of the problem to be studied

The purpose of this project was to investigate the use of a unique class of self-assembling crystalline nanostructures as reinforcing agents when incorporated as a minor component into polymeric composites. In particular, we were interested in self-assembling units comprised of cyclic peptides (8-mers) with alternating D- and L-amino acids. This scaffold had previously been shown to form nanotubular structures after solution-phase assembly, mediated by beta-sheet-like hydrogen bonding interactions that promoted stacking of the individual units. The rationale was that the chemical synthesis of these cyclic peptides could potentially yield a huge diversity in nanostructures by drawing upon the availability of Fmoc-protected amino acids with diverse side chains. These diverse structures could then be tailored to specific applications. At the time, a similar class of self-assembling nanostructured materials, based on the diphenylalanine scaffold, were mechanically characterized and declared to be the stiffest known organic material.1 This, in part, inspired our investigation into the mechanical properties of D-, L-cyclic peptides (DLCPs) and their potential application in reinforcing polymeric materials. The proposal was framed in the context of stabilizing load-bearing resorbable biomedical implants, like spinal fusion cages and bone screws, which are currently made from poly lactic acid and similar synthetic polymers, and sometimes fail prematurely and lead to poor healing outcomes for patients. However, if the surface chemistry of the DLCP nanotubes could be customized, there might be many applications where customizing polymer-filler interactions would be of utmost importance.

3) Summary of most important results

The actual term of the award lasted almost 12 months, during which time we were able to complete a preliminary investigation into the primary proposal goal. First, we synthesized a particular DLCP composed of alternating glutamine and leucine amino acids, QL4. This particular peptide was known to assemble from previous literature reports, but its micro-scale structure was ill-defined. We determined that the peptides formed polydispersed crystalline needle-like aggregates, with dimensions of ~1 micron (length) and ~100 nm (diameter), composed of longitudinally aligned nanotubes. These “microcrystals” could be isolated and co-dissolved with poly(D,L-lactic acid) (PDLLA) in organic solvents and then spun into microfibers using electrospinning. The resulting fibrous meshes contained the peptide microcrystals embedded in a polymer matrix. We tested the fibers by nanoindentation and found that the microcrystals increased the average stiffness of the fibers 5-fold. This work was published in Biomacromolecules.2

During the course of this investigation, we were intrigued by the mechanical properties of the microcrystals, given their ability to serve as filler materials in polymer composites. Therefore, after the award term concluded, we continued to build on our previous work to perform a more detailed study on the mechanical properties of the microcrystals themselves. This led to nanoindentation experiments and three-point bending experiments on the microcrystals, done with specialized instrumentation found in our collaborators’ lab in Singapore. The results of these experiments demonstrated that the DLCP microcrystals were comparable to the most mechanically robust proteinaceous materials known. This work was published in ACS Nano, and was made possible by the initial seed funding from ARO.3

Overall, the DLCP self-assembling system remains intriguing for their mechanical properties. However, we found that their assembly properties were much more adversely influenced by the peptide sequence than we originally hypothesized. For example, the DLCP composed of alternating leucine and glutamic acid forms interesting nano-scale tubular structures, but they were not robust enough to be harvested and incorporated into other fabrication protocols. Other DLCP sequences either did not assemble at all, despite exploring a wide variety of assembly conditions, or formed large aggregates that could not be resuspended in any solvents. Future work may explore conjugation of polymers to the amino acid side chains to increase processability of the nanotubes and further investigations of their biocompatibility.

4) Bibliography

Please refer to our publications for relevant references

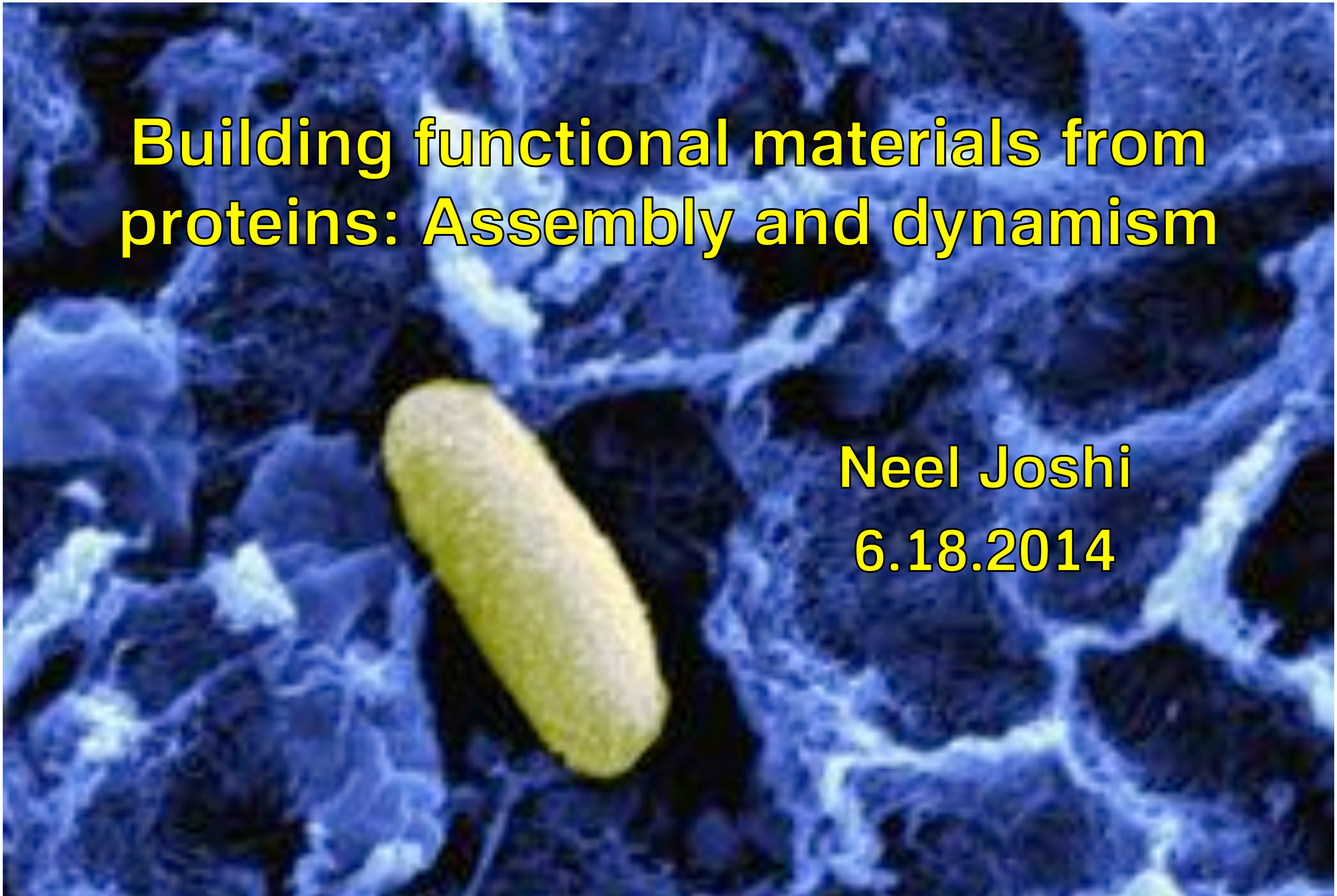
1. Even, N., Adler-Abramovich, L., Buzhansky, L., Dodiuk, H. & Gazit, E. Improvement of the Mechanical Properties of Epoxy by Peptide Nanotube Fillers. Small 7, 1007–1011 (2011).
2. Rubin, D. J. et al. Mechanical Reinforcement of Polymeric Fibers through Peptide Nanotube Incorporation. Biomacromolecules 14, 3370–3375 (2013).

3. Rubin, D. J. et al. Structural, Nanomechanical, and Computational Characterization of d, l-Cyclic Peptide Assemblies. ACS nano 9, 3360–3368 (2015).

Technology Transfer

The PI traveled to give invited talks at The ARL lab in Aberdeen, MD and the AFOSR lab in Dayton, OH. Slides from the talks are included as an attachment.

Building functional materials from proteins: Assembly and dynamism

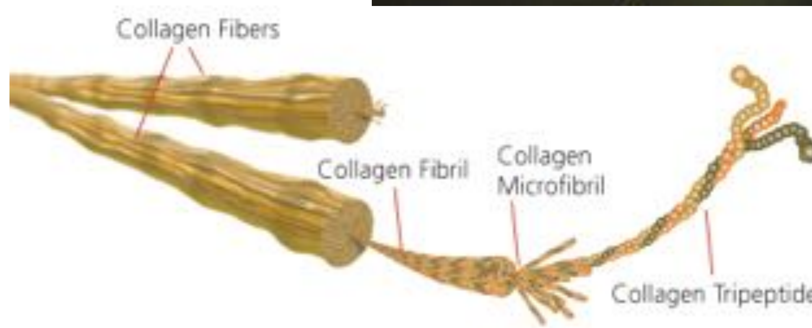


Neel Joshi
6.18.2014

HARVARD
School of Engineering
and Applied Sciences

WYSS INSTITUTE

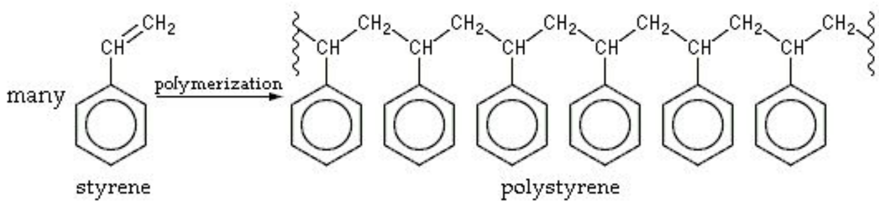
Living systems use proteins to build



SILKS

VERTEBRATE TISSUES

Synthetic Polymers

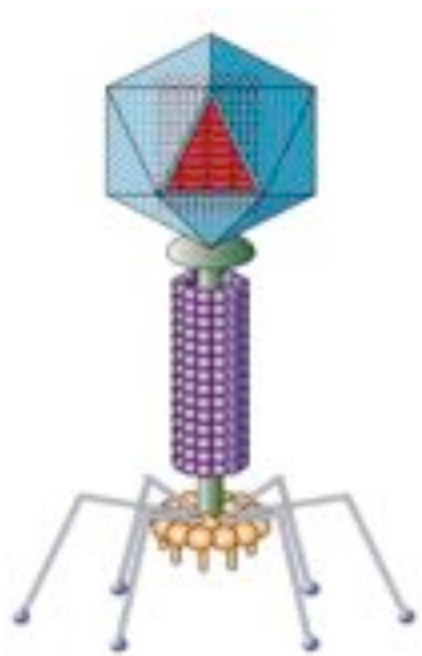


Limited sequence control

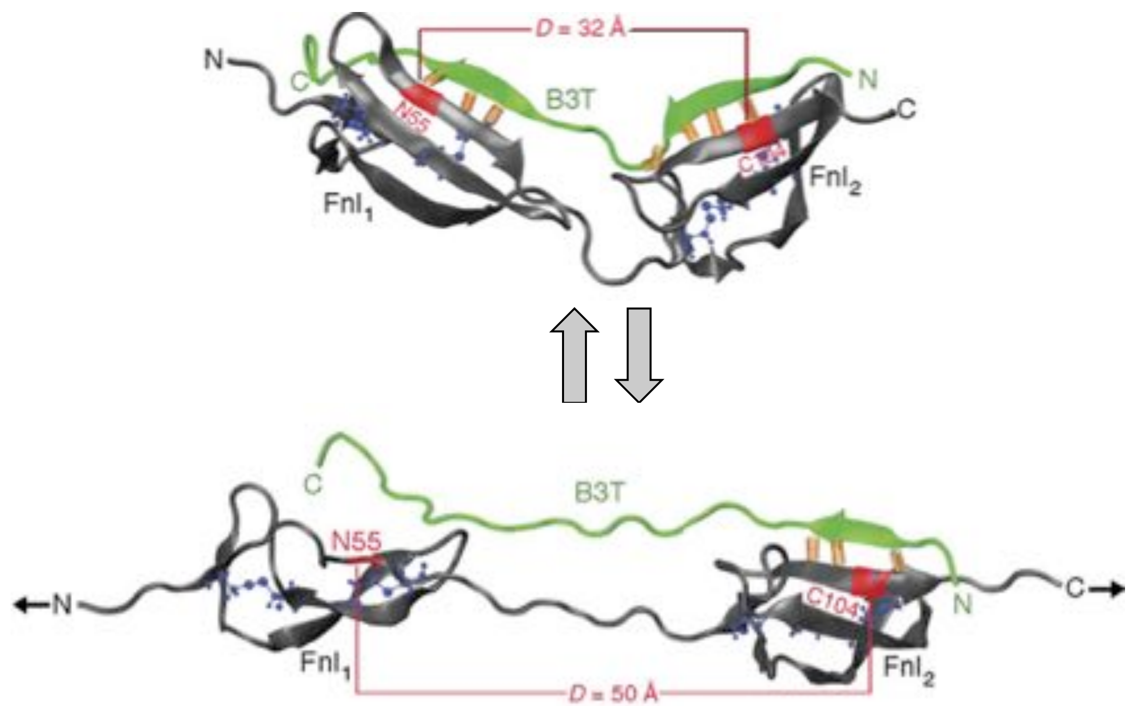
Proteins

Complete sequence control

Self-assembly

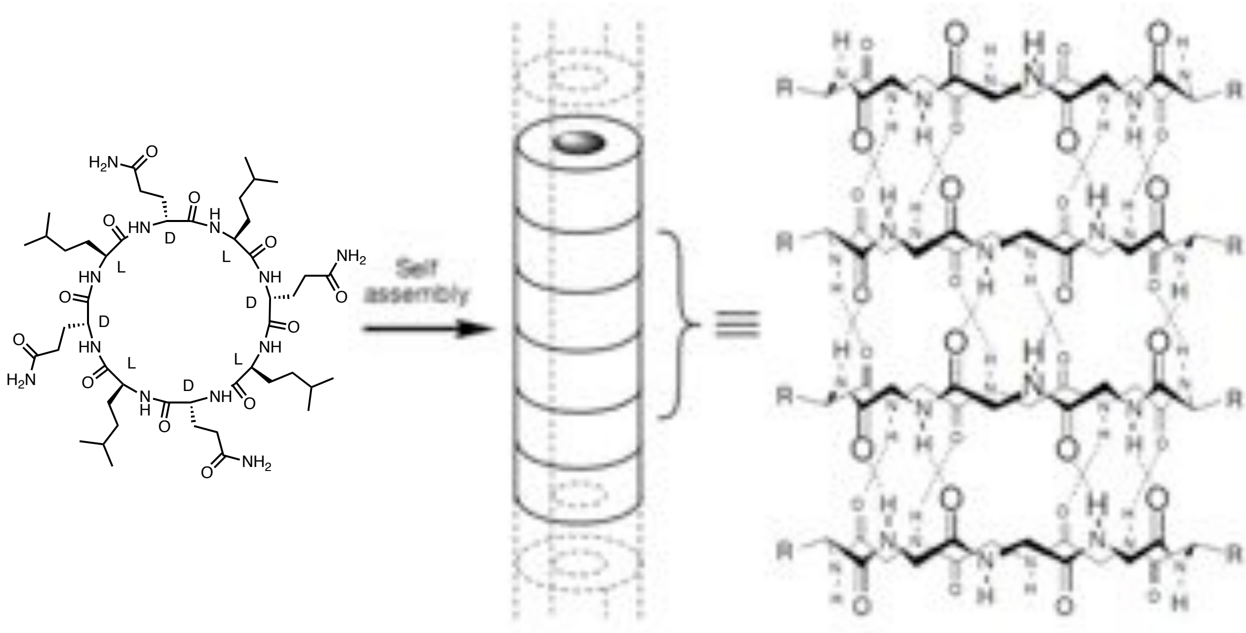


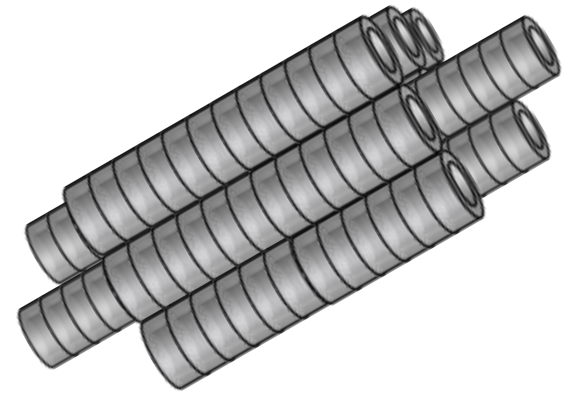
Structural Dynamism

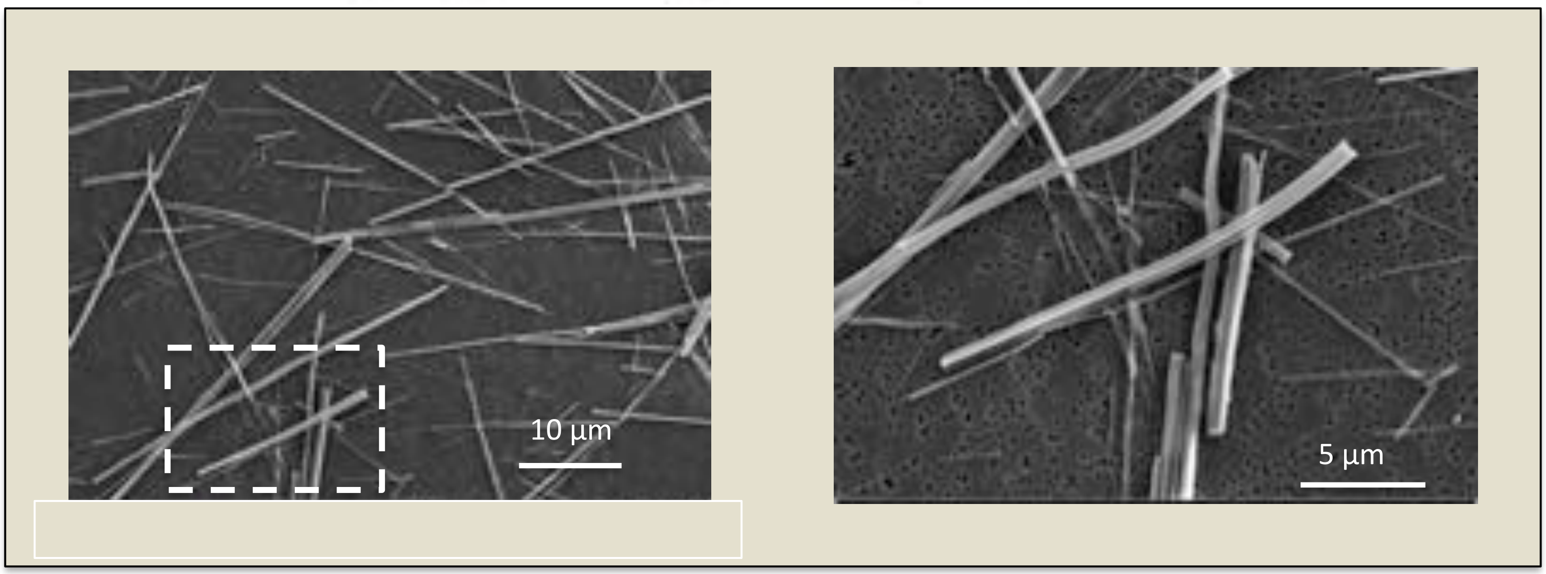


peptides -----> proteins -----> networks of proteins

D,L-cyclic peptides (DLCPs)

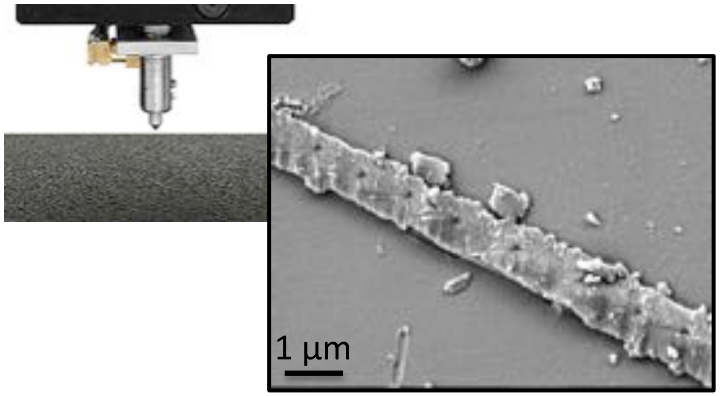




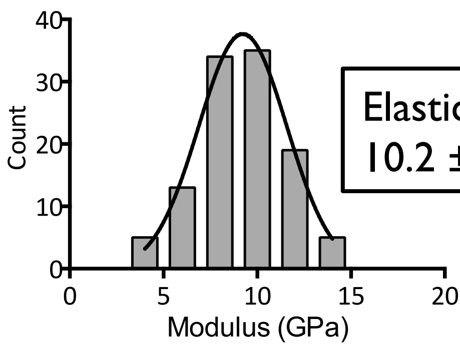


Ghadiri, et al. *Nature* **1993**

DLCPs are remarkably stiff

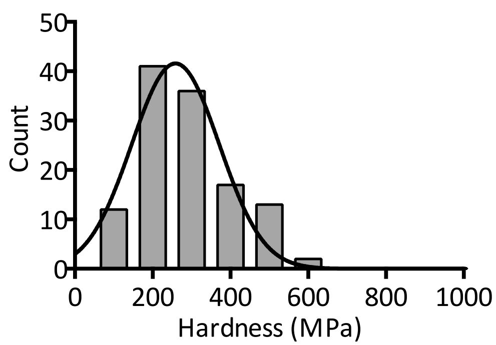


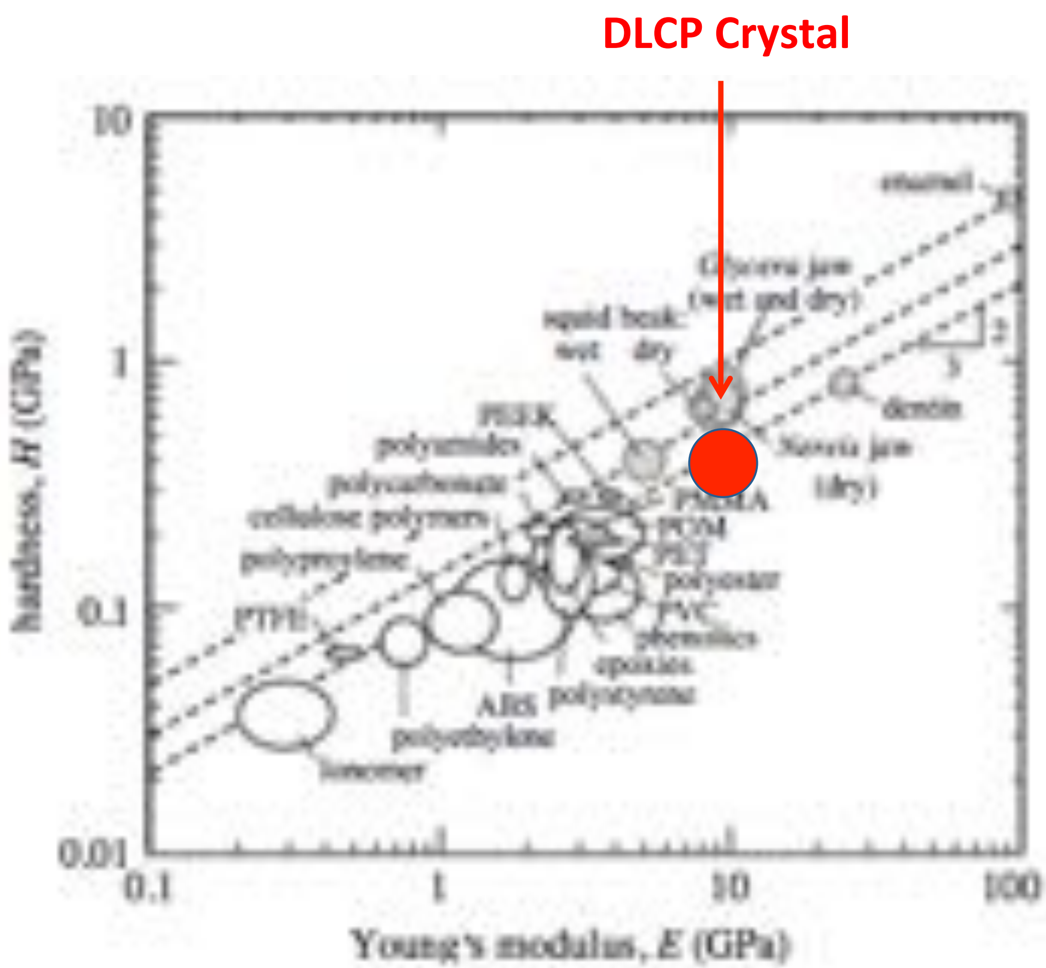
N = 111



Elastic Modulus = 10.2 ± 0.5 GPa

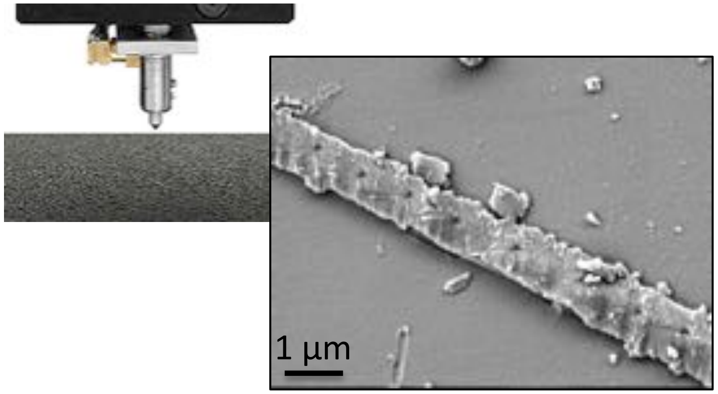
Hardness = 343 ± 12 MPa



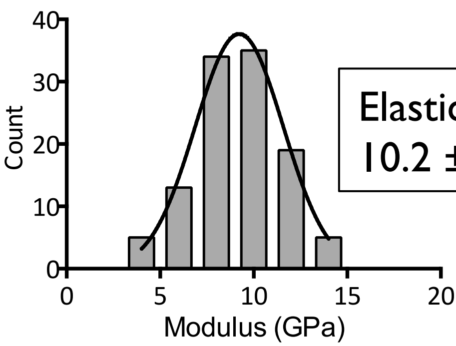


Dan Rubin

DLCPs are remarkably stiff

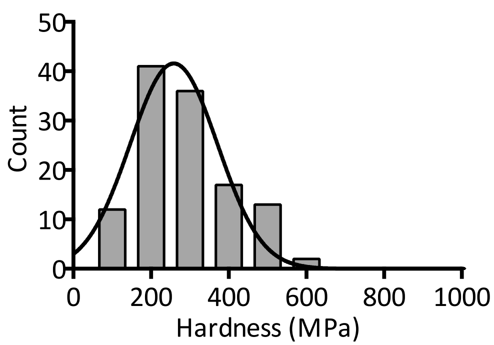


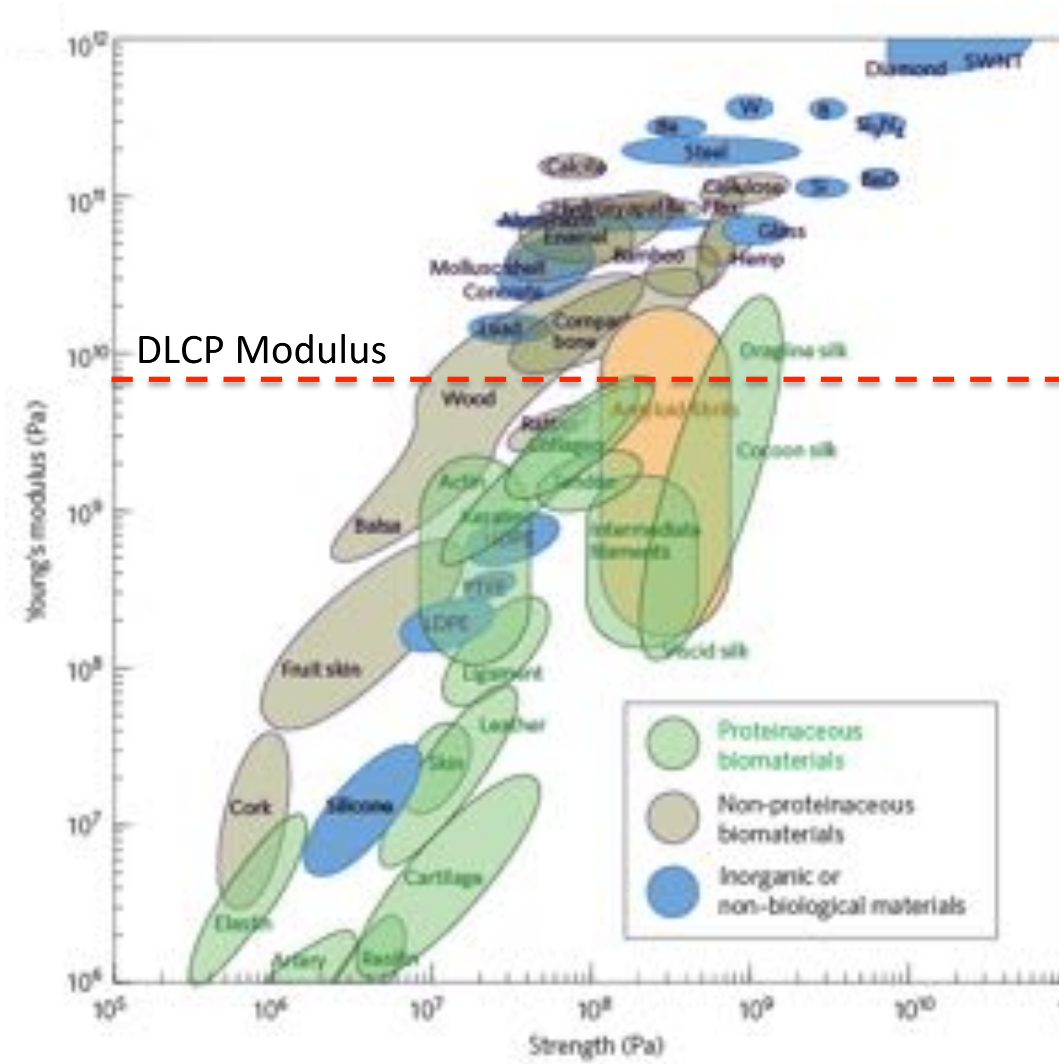
N = 111



Elastic Modulus = 10.2 ± 0.5 GPa

Hardness = 343 ± 12 MPa



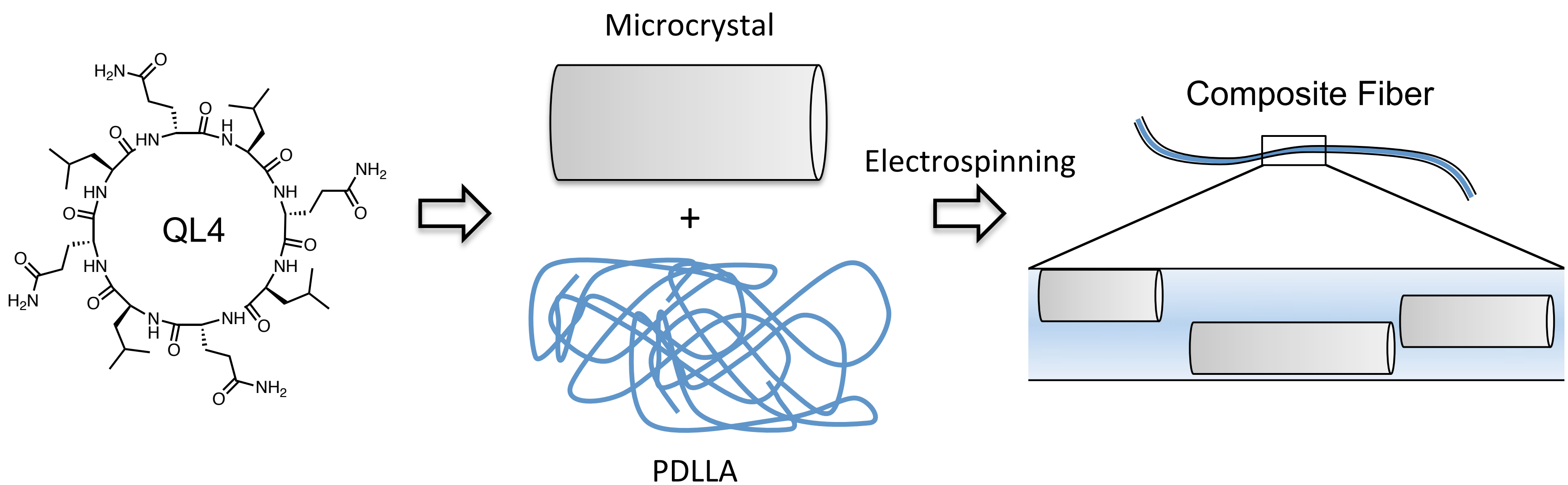


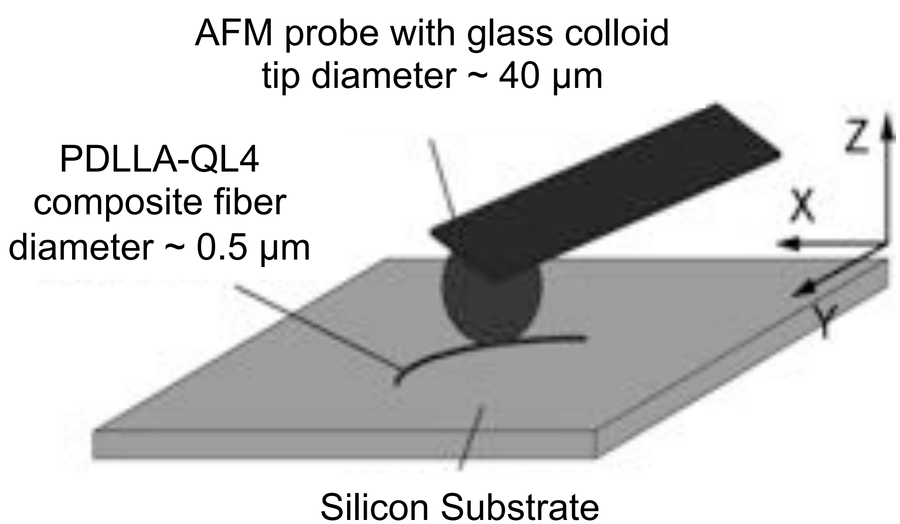
HARVARD School of Engineering and Applied Sciences

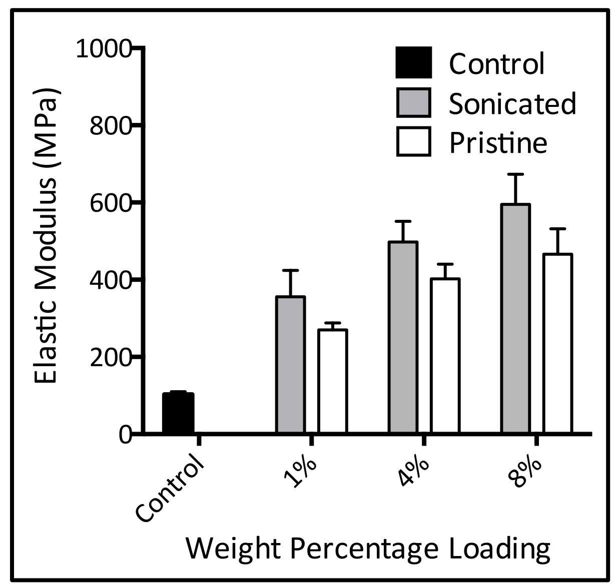
WYSS INSTITUTE

Knowles, T. et al. *Nature Nanotech.* (2011) 6, 469-479

DLCPs can reinforce polymeric fibers

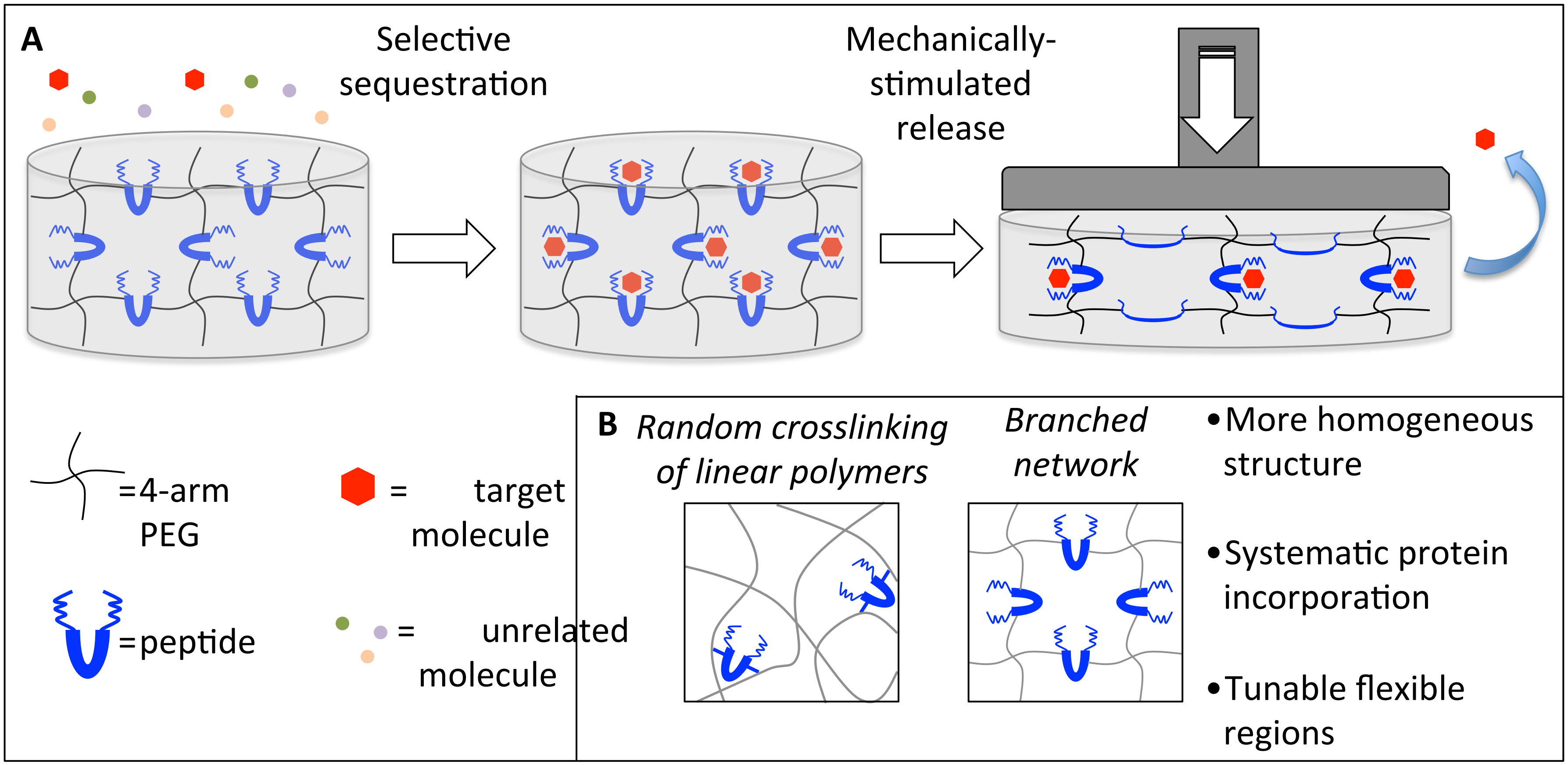






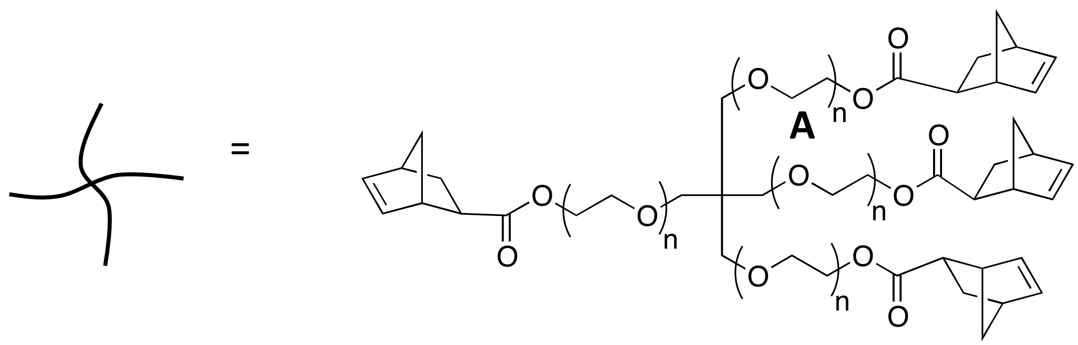
peptides ----> proteins ----> networks of proteins

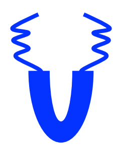
Mechano-sensitive protein-polymer hybrids



Mechano-sensitive protein-polymer hybrids

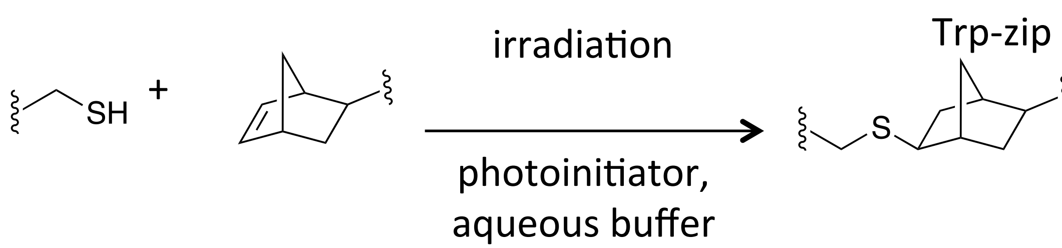
Components for gelation reaction



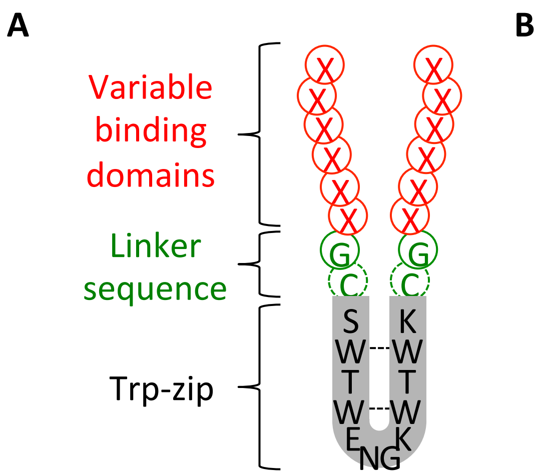


= HANFFQCGS**W**T**W**ENGK**W**T**W**KCGWKYNQS

Thiol-ene reaction

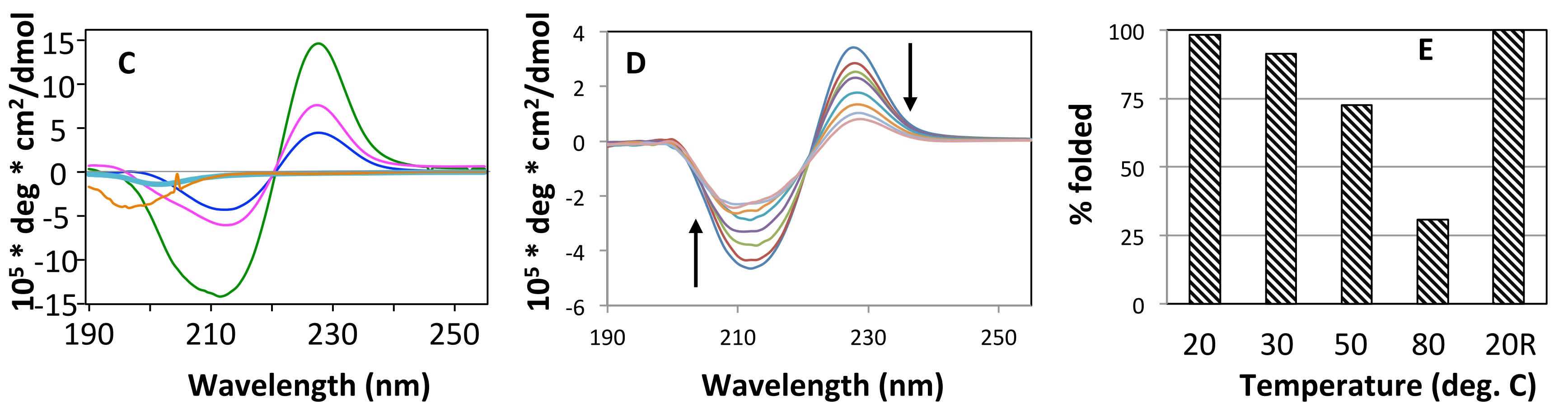


Mechano-sensitive protein-polymer hybrids



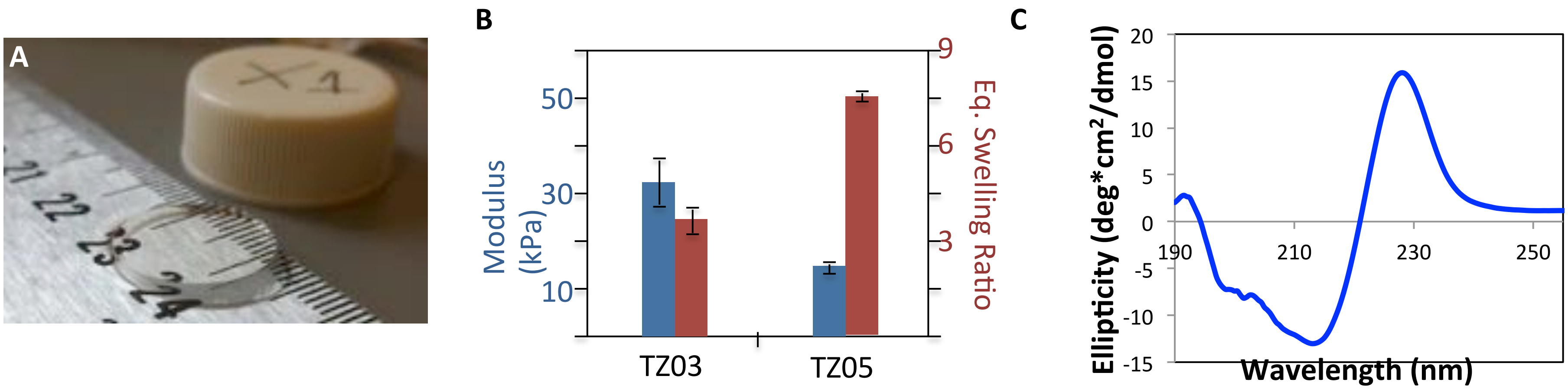
B

Name	Sequence	Forms β-turn	Forms gels	Binds VEGF
TZ01	SWTWENGKWTWK	✓	✗	✗
TZ02	HANFFQGSWTWENGKWTWKGWKYNQS	✓	✗	✓
TZ03	CGSWTWENGKWTWKCG	✓	✓	✗
TZ04	HANFFQCGSWTWENGKWTWKCGWKYNQS	✓	✓	✓
TZ05	CGSATAENGKATAKCG	✗	✓	✗
TZ06	HANFFQCGSATAENGKATAKCGWKYNQS	✗	✓	✗



HARVARD School of Engineering and Applied Sciences WYSS INSTITUTE

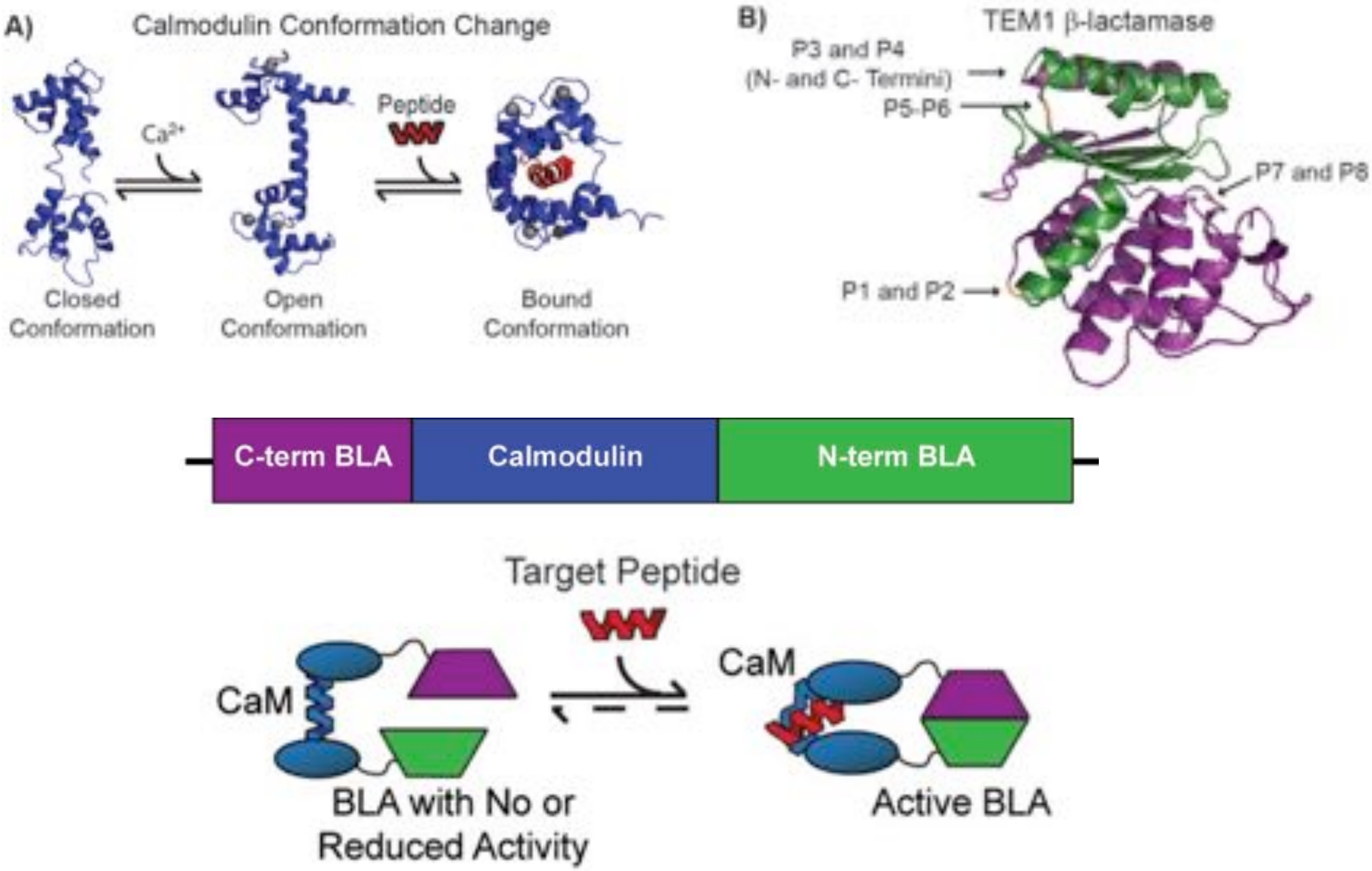
Mechano-sensitive protein-polymer hybrids



HARVARD School of Engineering and Applied Sciences

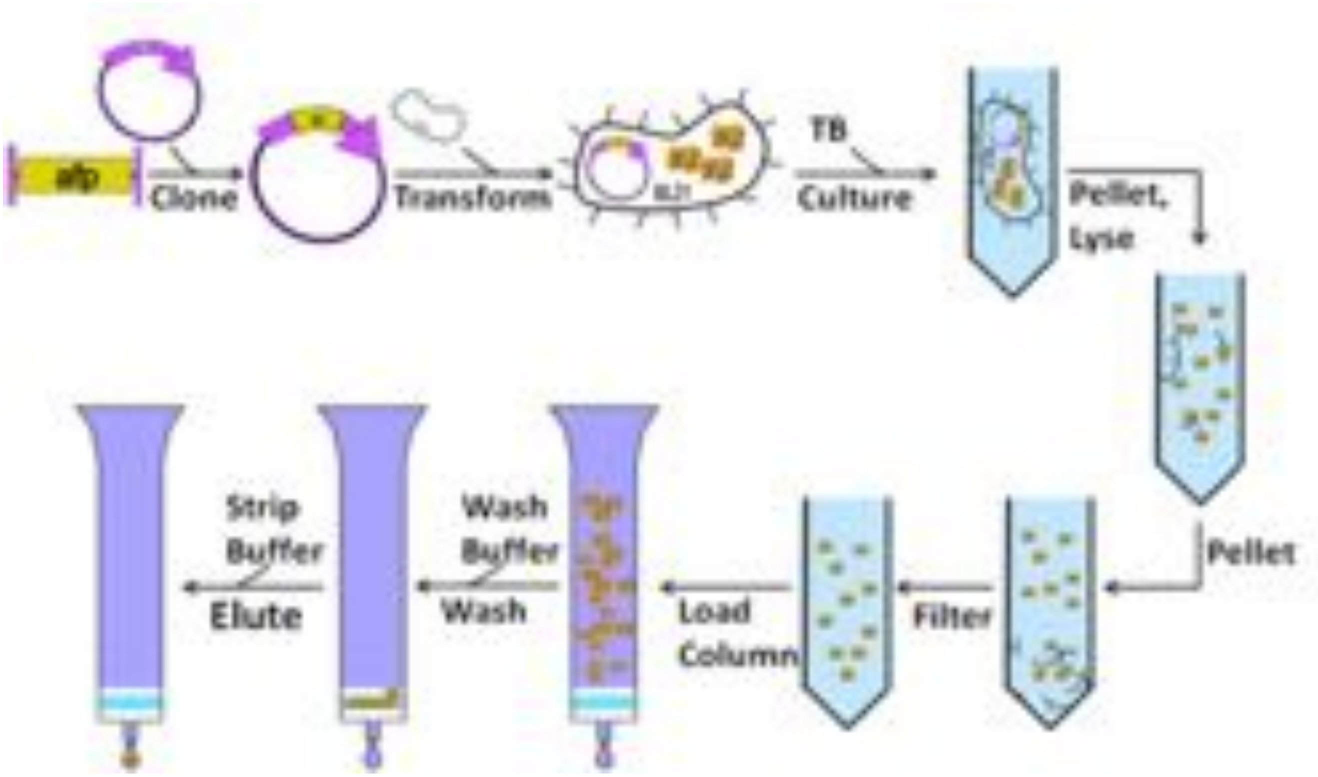
WYSS INSTITUTE

Biosensing Platforms Based on Protein Conformational Dynamics



Meister, et al. *ChemBioChem* **2013** 14(*12*), 1460-1467 peptides -----> proteins -----> networks of proteins

Engineered protein manufacturing



Compared to biological materials:

- Difficult to obtain pure and in large quantities
- Time consuming, less cost effective
- Not appropriate for large-scale materials

Materials fabrication

Material design parameters

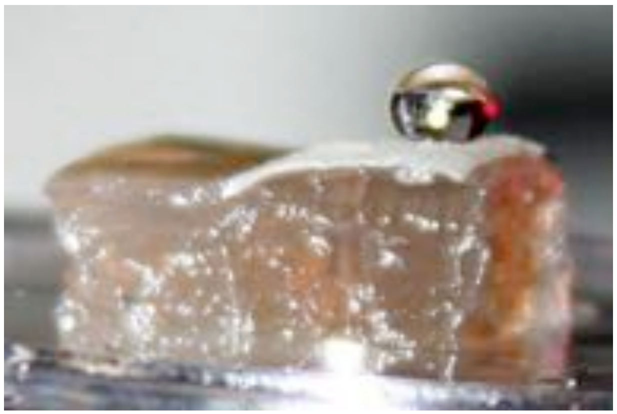
1) Must be able to control sequence using conventional genetic engineering

2) Must be able to produce material on large scales by harnessing biosynthetic potential of a living organism

3) No protein purification

Biofilms

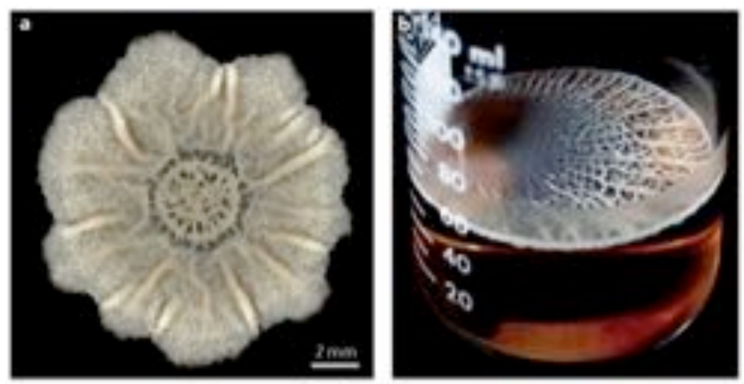
Self-standing, macroscopic, biosynthetic materials



Epstein, et al. *PNAS* **2010**

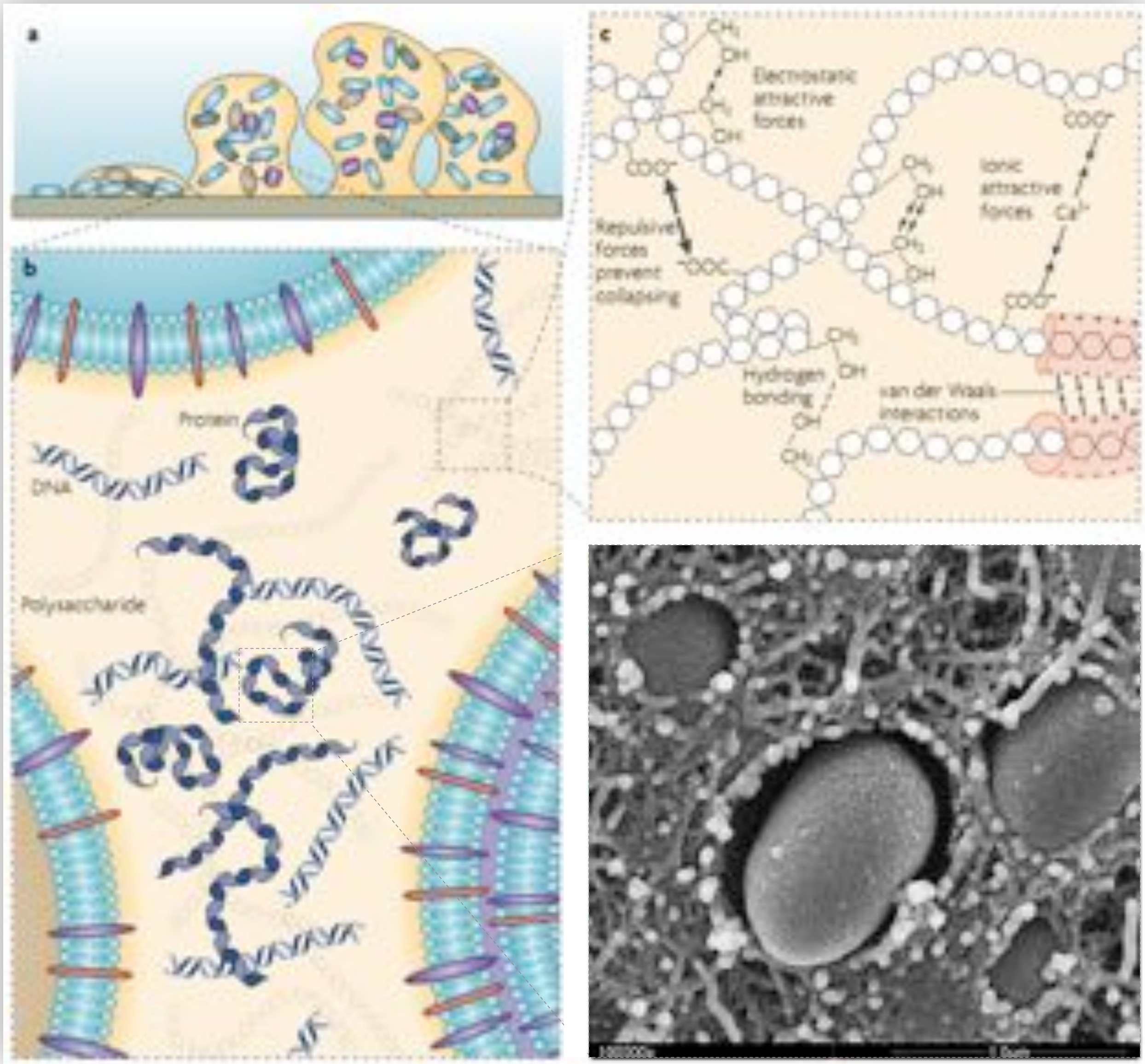
http://www.biofilm.montana.edu/node/2390

weitzlab.seas.harvard.edu



Vlamakis, et al. *Nat. Rev. Microbiol.* **2013**

Biofilm Nano-architecture



Nature Rev. Microb. 2010, 8:623.

mBio. 2013, 4(2):e00103-13

Domesticating the Microbe

1 2

3

4

Bacteria are dangerous!!!
(Germ Theory of Disease)
Pasteur and Koch, early 19th century

We understand how they work…
(Antibiotics, Microbiology, Molecular Biology)
Fleming, Watson, Crick , Lederberg, Brenner…et al.

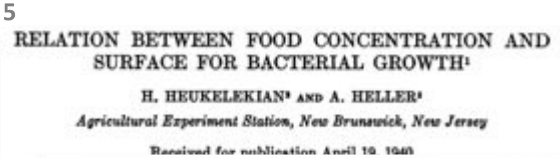
We can exploit them.
(Recombinant DNA Technology)
Cohen, Boyer, and Lobban

Biofilms are bad!!!

We are starting to understand how they work…

***Can* we exploit them?**

5



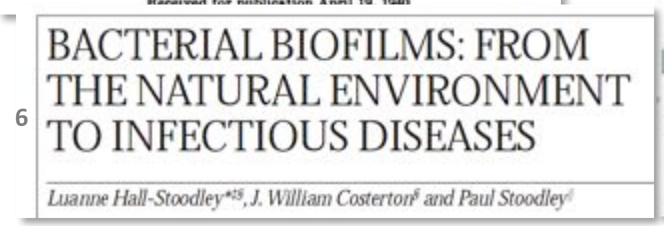
RELATION BETWEEN FOOD CONCENTRATION AND SURFACE FOR BACTERIAL GROWTH[1]

H. HEUKELEKIAN[2] AND A. HELLER[3]

Agricultural Experiment Station, New Brunswick, New Jersey

Received for publication April 19, 1940

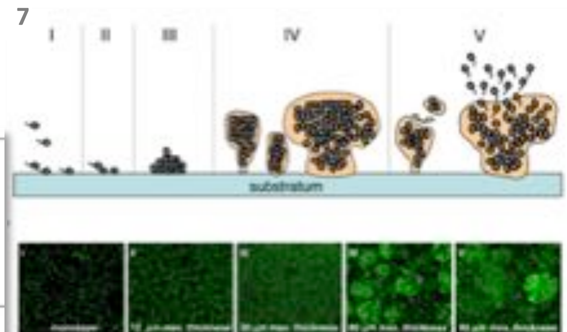
6



BACTERIAL BIOFILMS: FROM THE NATURAL ENVIRONMENT TO INFECTIOUS DISEASES

Luanne Hall-Stoodley[*‡§], J. William Costerton[§] and Paul Stoodley[||]*

7



8

1,2. Wikimedia Commons, 3. Time Magazine, Inc., 4. Genentech, 5. *J. Bacteriol.*, 1940. **40**(4):547, 6. *Nat. Rev. Microb.* 2004.**2**, 95-108, 7. Clin Rev Allergy Immunol, 2008. **35**(3): 124, 8. Lower Lab, OSU.

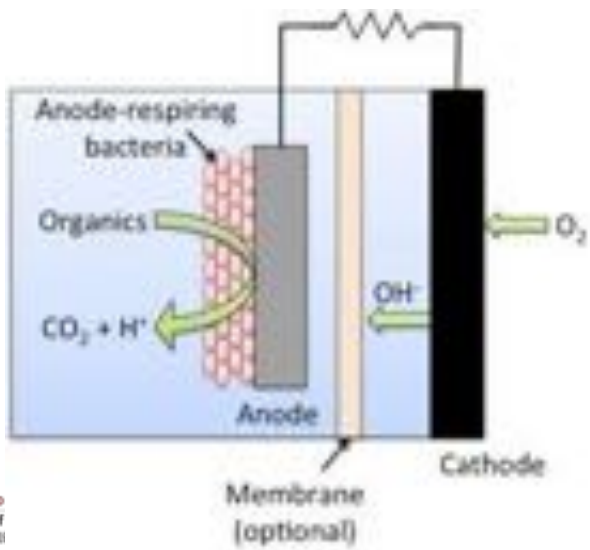
HARVARD School of Engineering and Applied Sciences WYSS INSTITUTE

Industrial Biofilm Usage

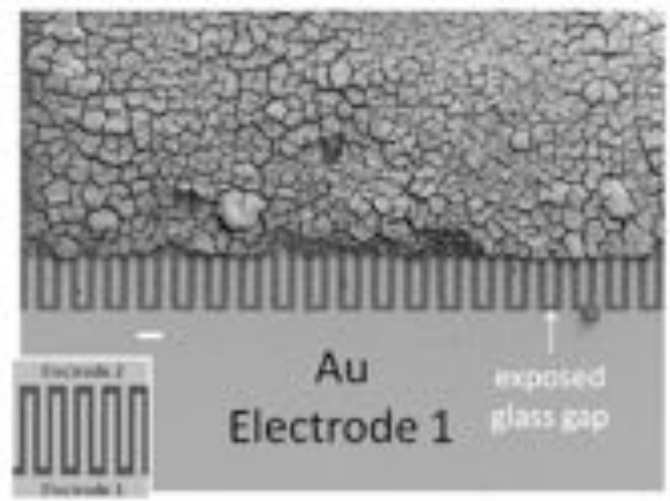
Wastewater treatment

Chemical processing

Microbial Fuel Cells

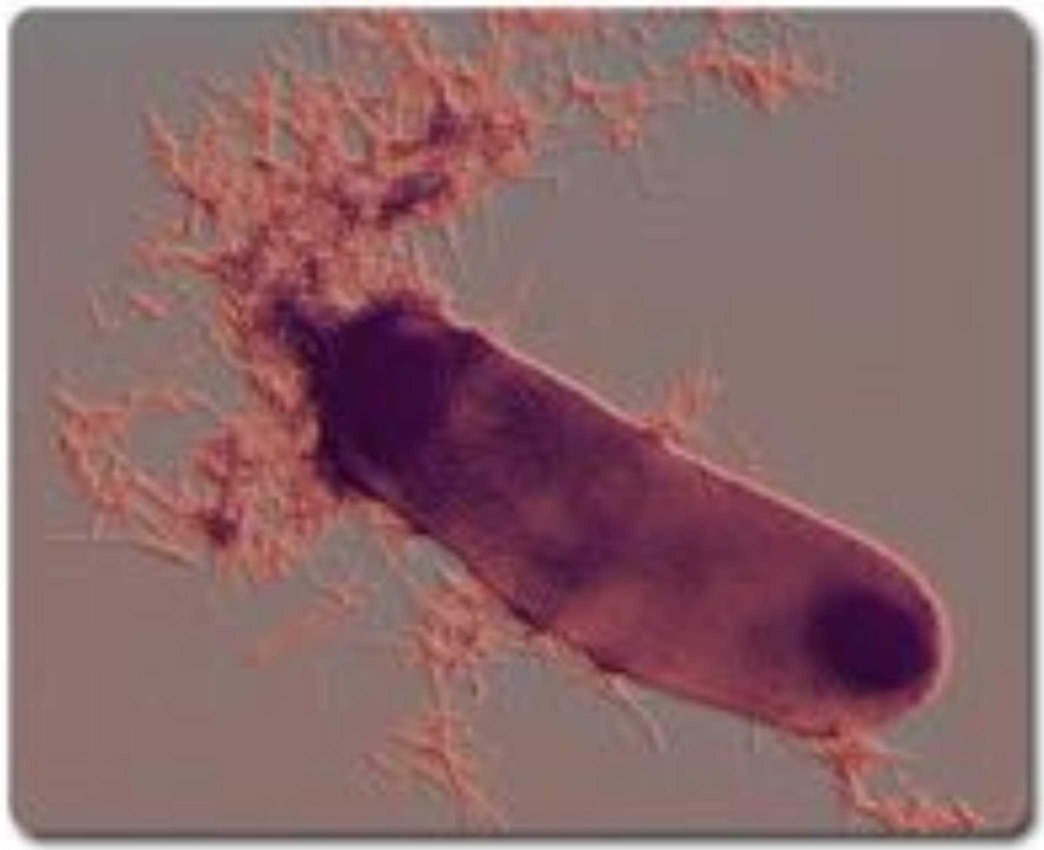


Microbial circuits



Snider, R. M. et al. PNAS. 2012, 15467.

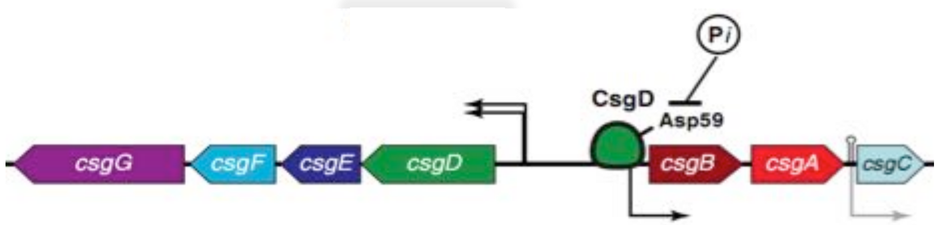
Bacterial ECM Proteins: Functional Amyloids

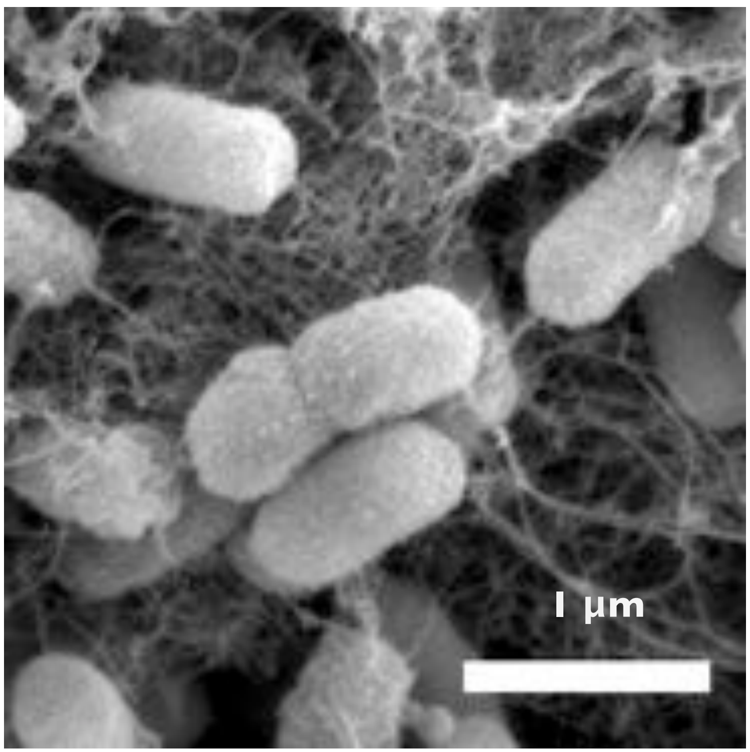


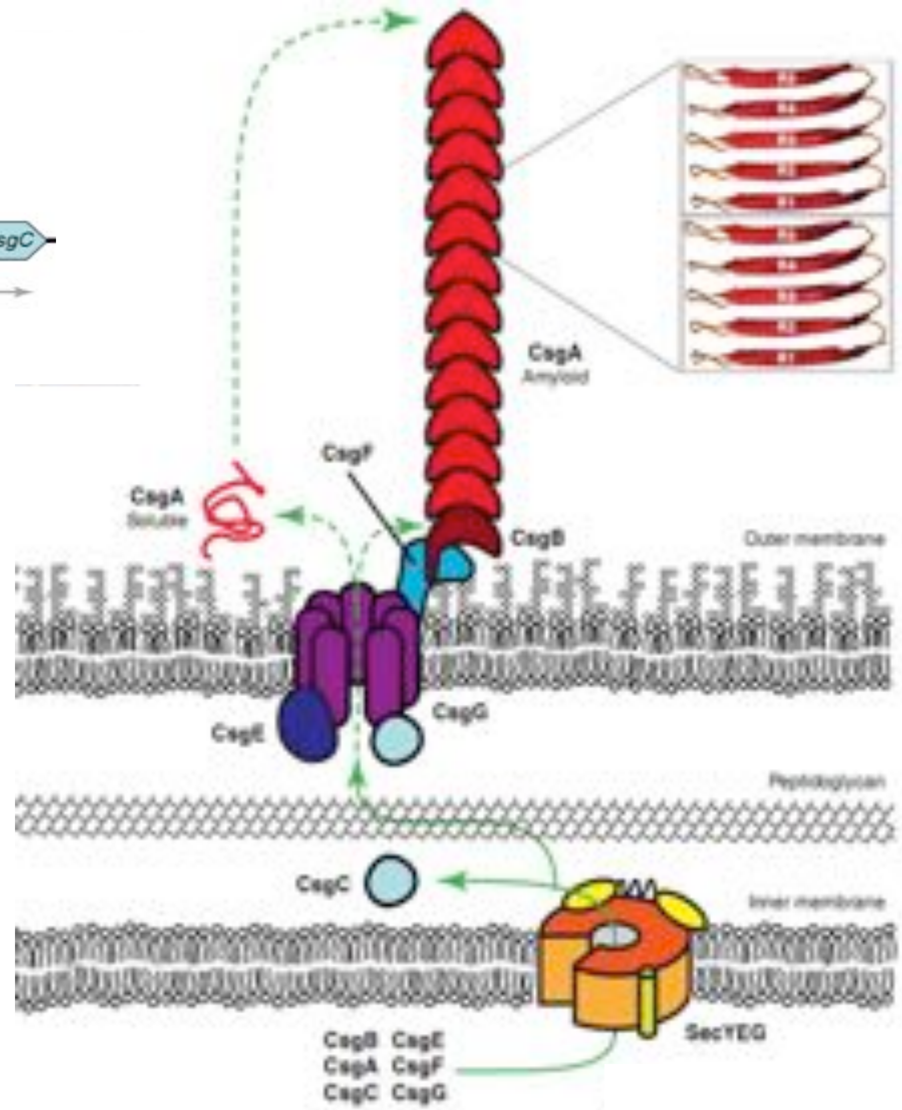
http://labs.mcdb.lsa.umich.edu/labs/chapman/

- Mediate adhesion to surfaces
- Can be up to 60% of biofilm biomass

E. Coli: Curli Biosynthesis



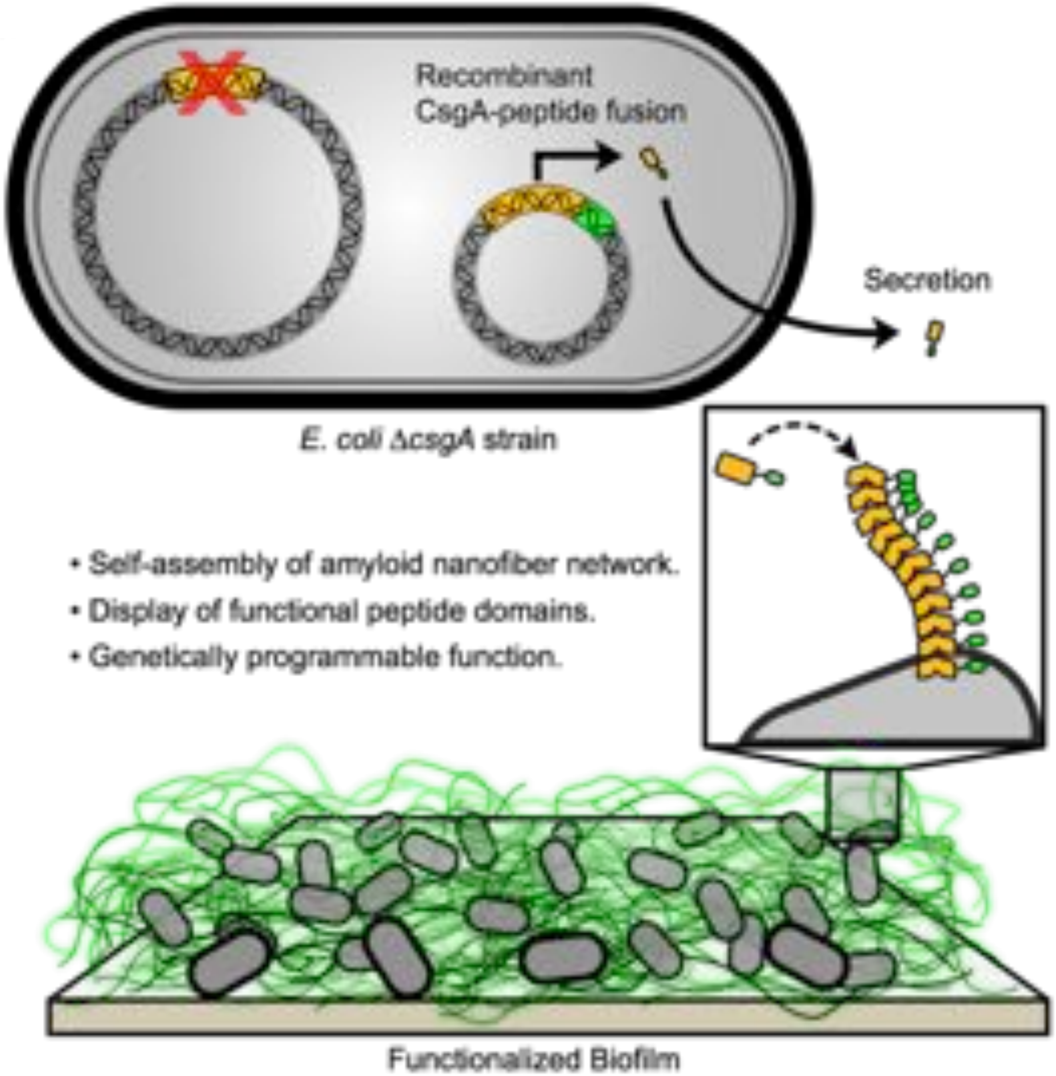




Trends in Microbiology February 2012, Vol. 20, No. 2

The BIND Concept

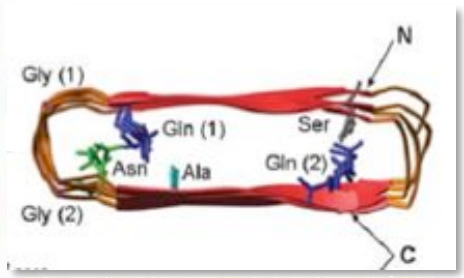
Biofilm-Integrated Nanofiber Display



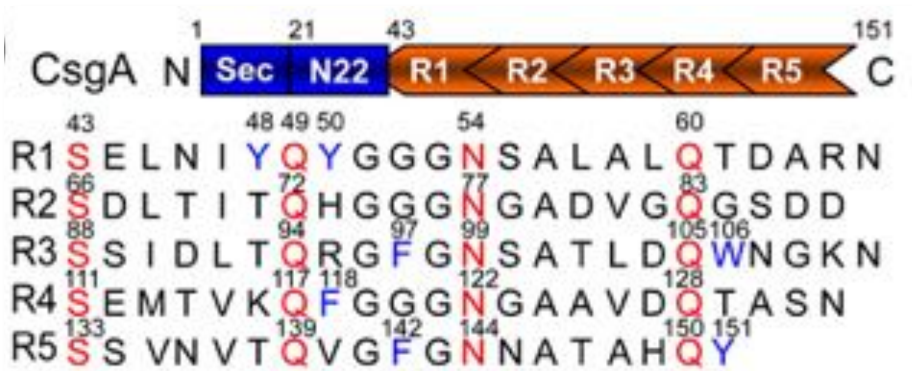
- Nanofibers have diameters from 4-7nm and are tens of microns in length.
- Nanofiber network is robust
- Amyloids: strength comparable to steel and stiffnesses comparable to silk

HARVARD School of Engineering and Applied Sciences WYSS INSTITUTE

CsgA Protein Structure



Annu. Rev. Microbiol. 2006. 60:131–47

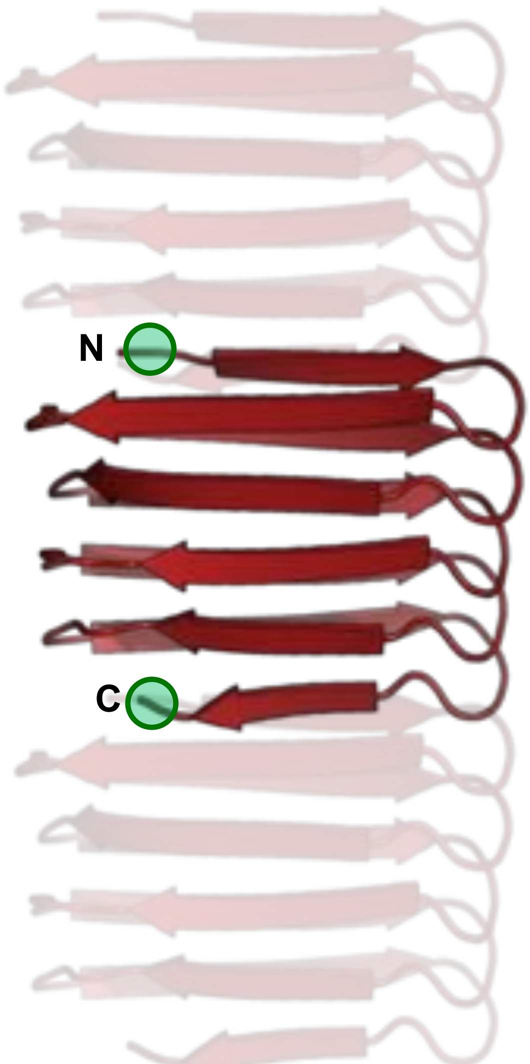


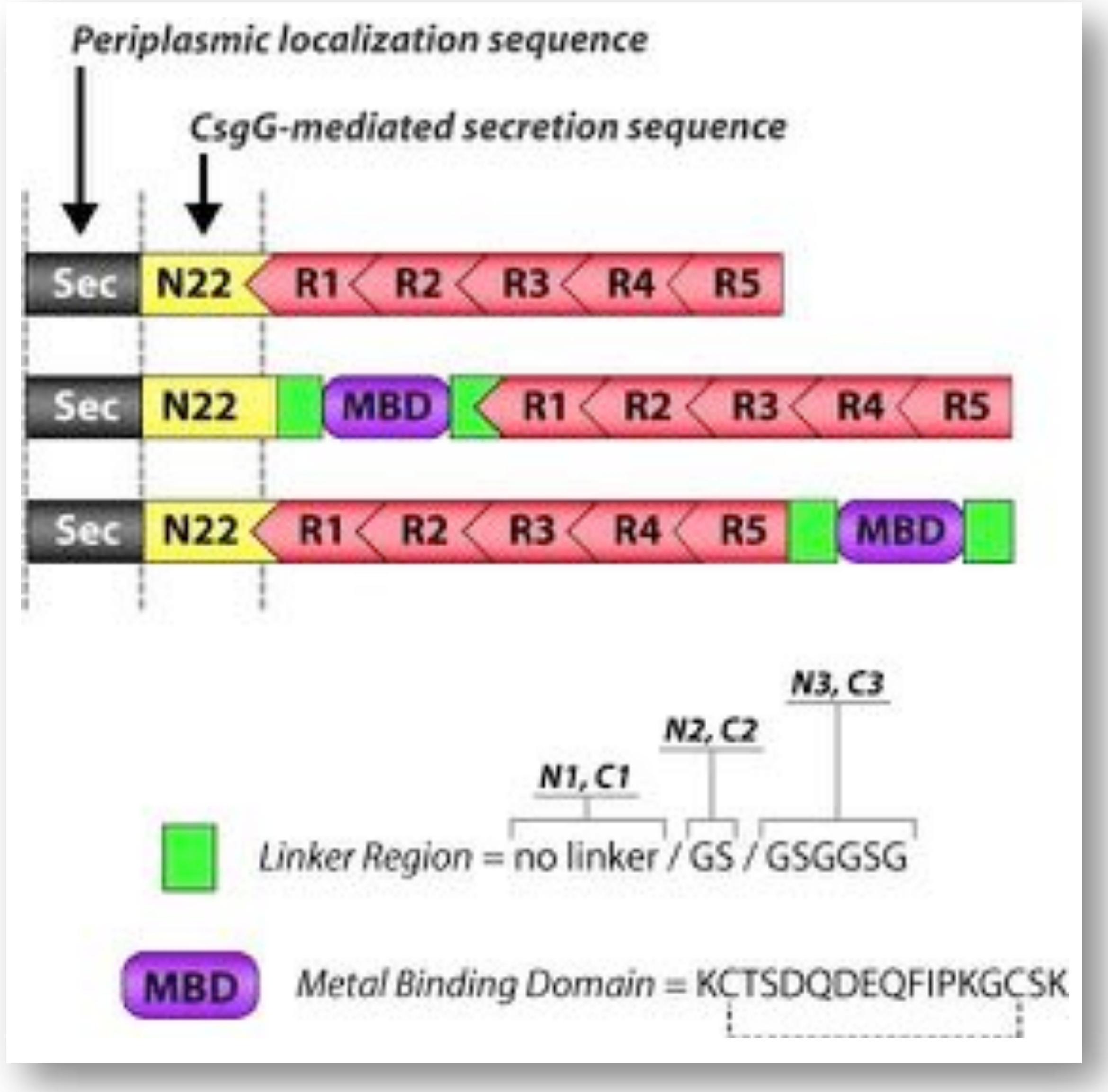
J. Mol. Biol. (2008) **380**, 570–580

- Assembled from the secreted CsgB protein (17.5kDa) which is membrane-anchored.
- Easily detected using Congo Red, which stains amyloid fibers.
- Assembly kinetics can be monitored *in vitro* by Thioflavin T.

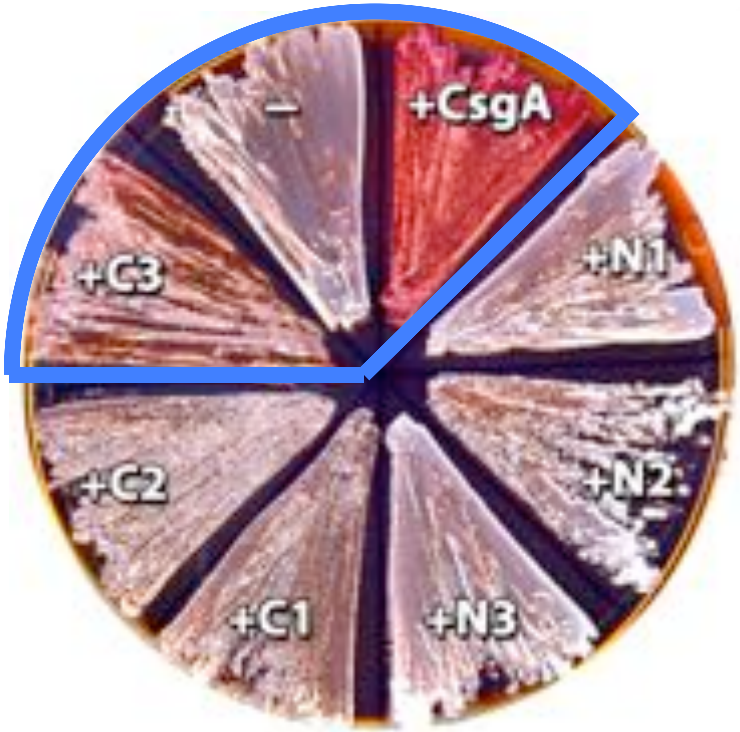
HARVARD School of Engineering and Applied Sciences WYSS INSTITUTE

CsgA Insertion Library



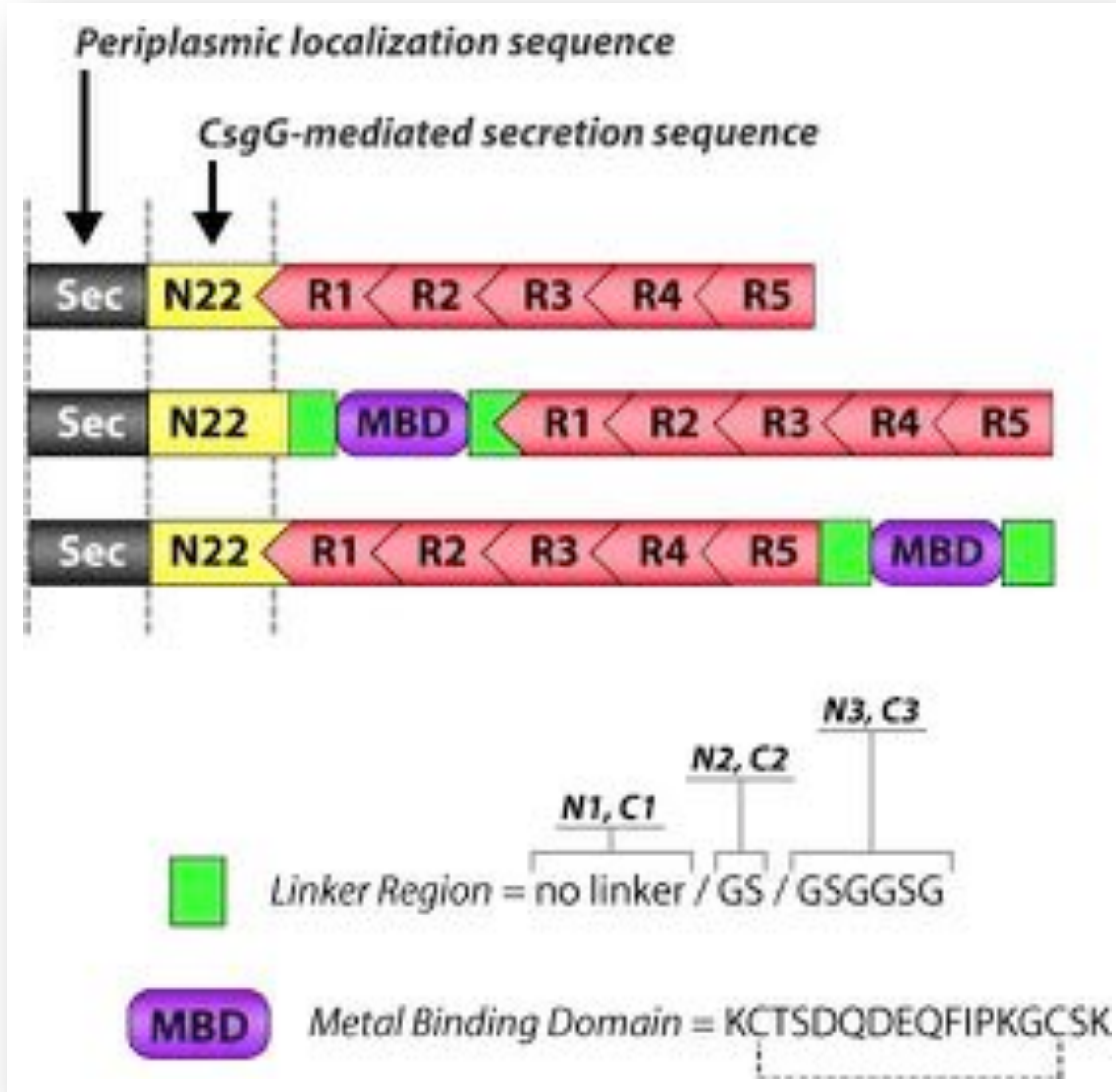


CsgA Insertion Library

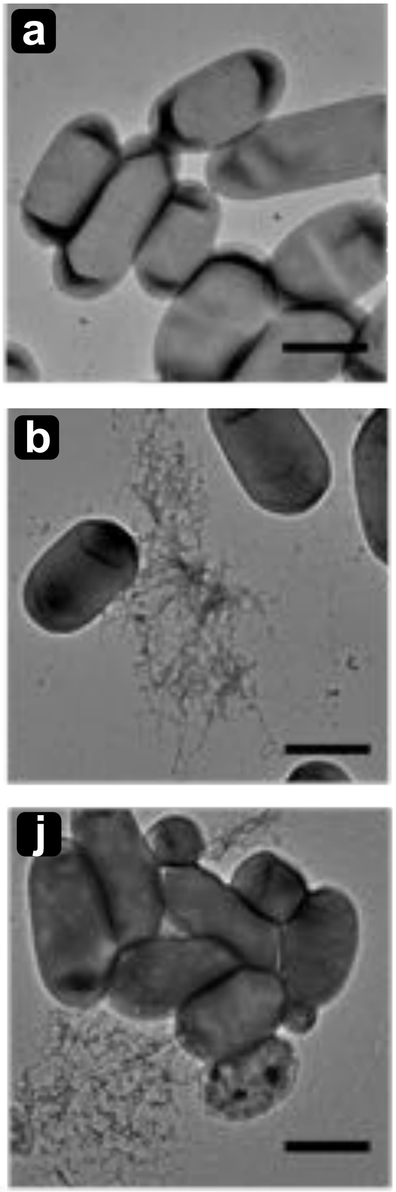


Congo Red assay

Red = amyloid formation



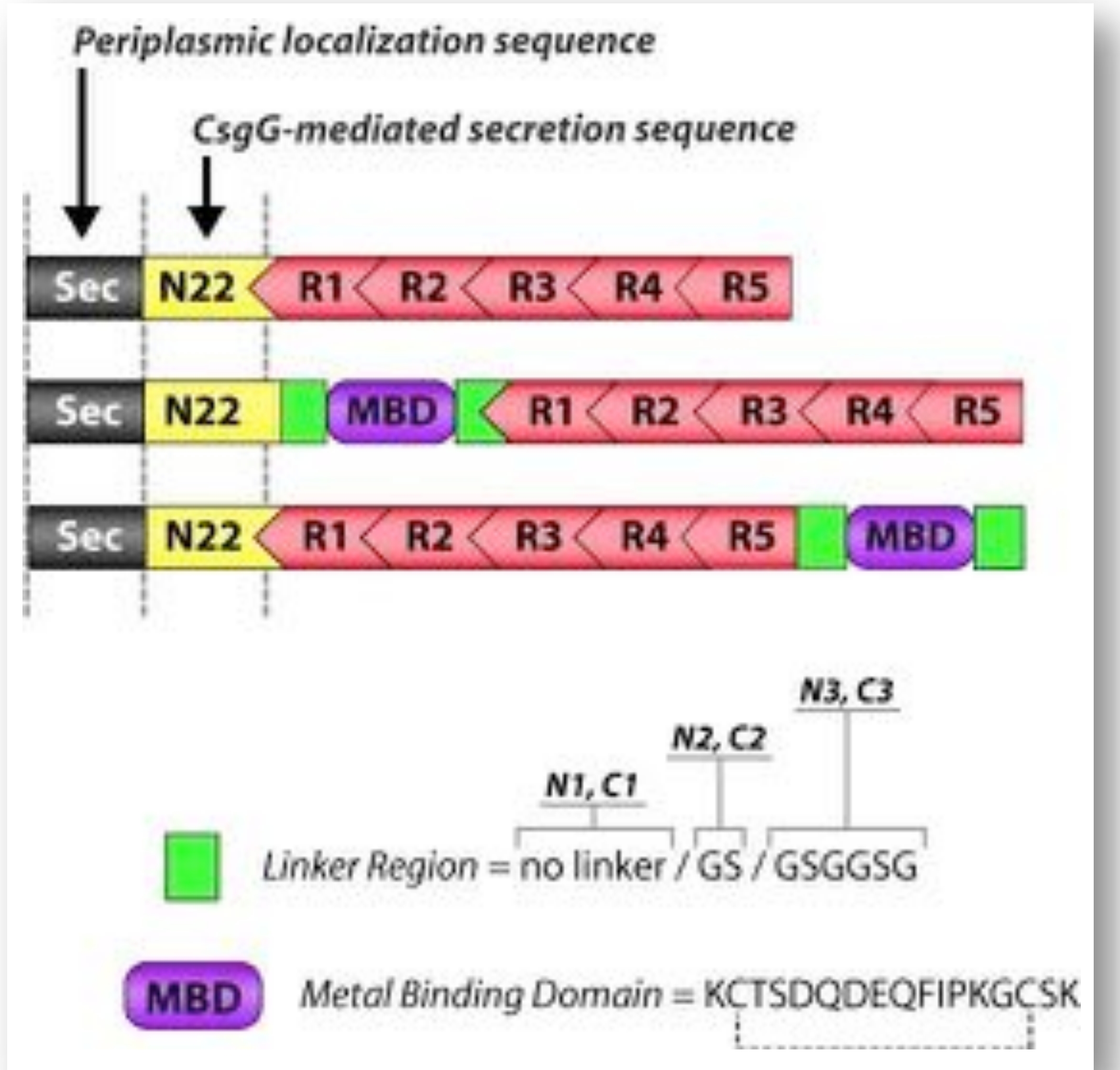
CsgA Insertion Library



no CsgA (–)

wt-CsgA (+CsgA)

CsgA-MBD (C3)



HARVARD School of Engineering and Applied Sciences

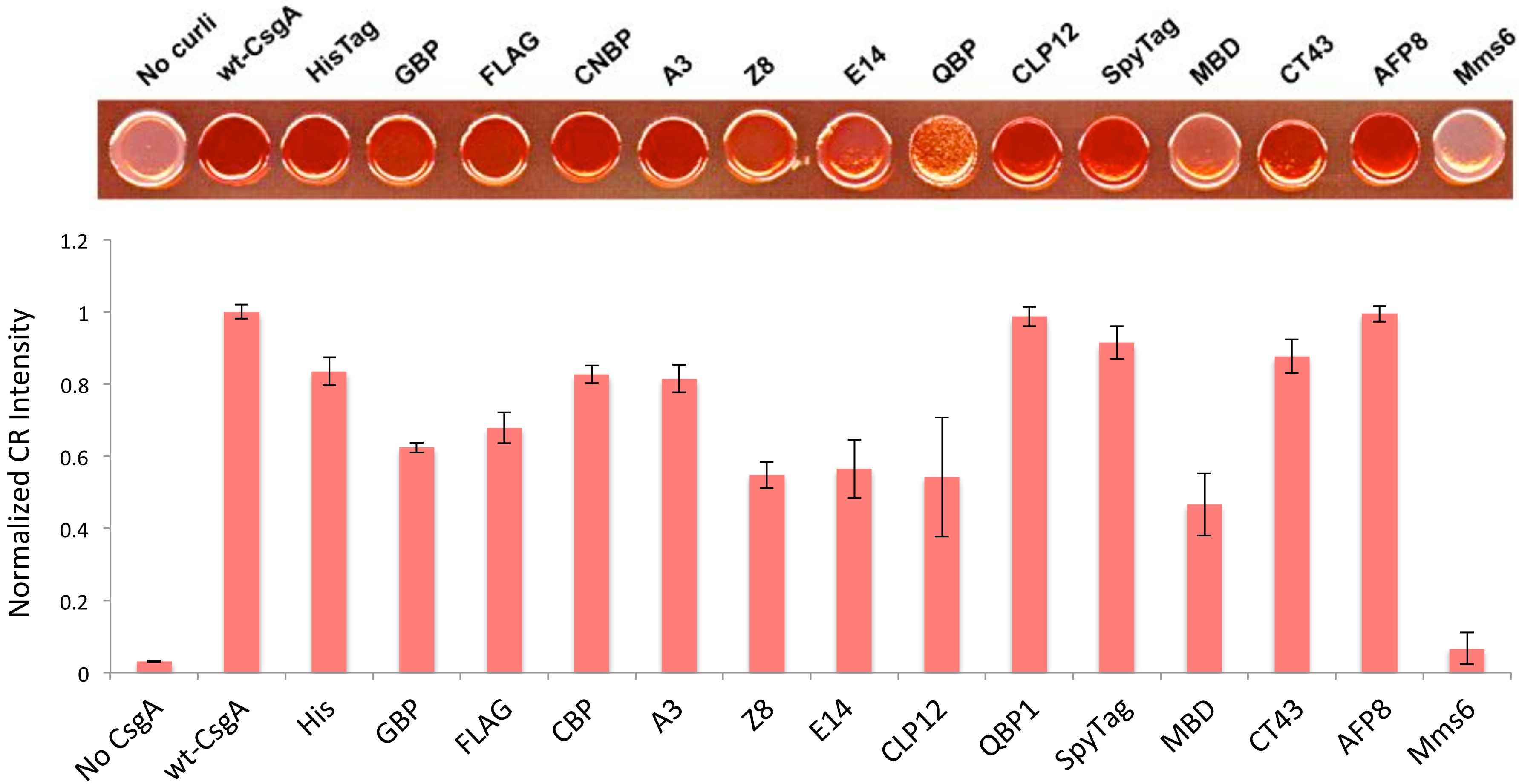
WYSS INSTITUTE

Peter Nguyen

CsgA-peptide Insertion Library

Peptide	Sequence	Length (aa)	Type	Function	Reference
HIS	HHHHHH	6	Affinity Tag	Affinity Tag	Bio/Technology 1988, 6(11): 1321.
GBP	EPLQLKM	7	Substrate Binding	Graphene edge binding	JACS 2011, 133: 14480.
FLAG	DYKDDDDK	8	Affinity Tag	Affinity Tag	Nature Biotech. 1988, 6: 1204.
CNBP	HSSYWYAFNNKT	12	Substrate Binding	Carbon nanotube binding	Nano. Lett. 2006, 6: 40.
A3	AYSSGAPPMPPF	12	Substrate Binding	Gold surface binding	Small 2005, 1(11): 1048.
Z8	LRRSSEAHNSIV	12	NP templating	ZnS quantum dot templating	J. Mater. Chem. 2003, 13: 2414.
E14	PWIPTPRPTFTG	12	NP templating	CdS quantum dot templating	J. Mater. Chem. 2003, 13: 2414.
CLP12	NPYHPTIPQSVH	12	Mineral templating	Hydroxyapatite nucleation	Langmuir 2011, 27: 7620.
QBP1	PPPWLPYMPPWS	12	Substrate Binding	Quartz/Glass binding	Bioinformatics 2007, 23: 2816.
SpyTag	AHIVMVDAYKPTK	13	Protein Display	General covalent capture/display of proteins	PNAS 2012, 109(12): E690.
BCCP	GLNDIFEAQKIEWH	14	Protein Display	Biotinylation tag	Prot. Sci. 1999, 8: 921.
MBD	KCTSDQDEQFIPKGCSK	17	Substrate Binding	Binding to stainless steel surfaces	Mol. Microb. 2006, 59(4): 1083.
CT43	CGPAGDSSGVDSRSVGPC	18	NP templating	ZnS quantum dot templating	JACS 2010, 132: 4731.
AFP8	DTASDAAAAAALTAANAKAAAE LTAANAAAAAAATAR	37	Substrate Binding	Ice crystal binding	JBC 1998, 273(19): 11714.
Mms6	GGTIWTGKGLGLGLGLGLGAW GPIILGVVGAGAVYAYMKSRDI ESAQSDEEVELRDALA	59	NP templating	Magnetite NP templating	JBC 2003, 278(10): 8745.

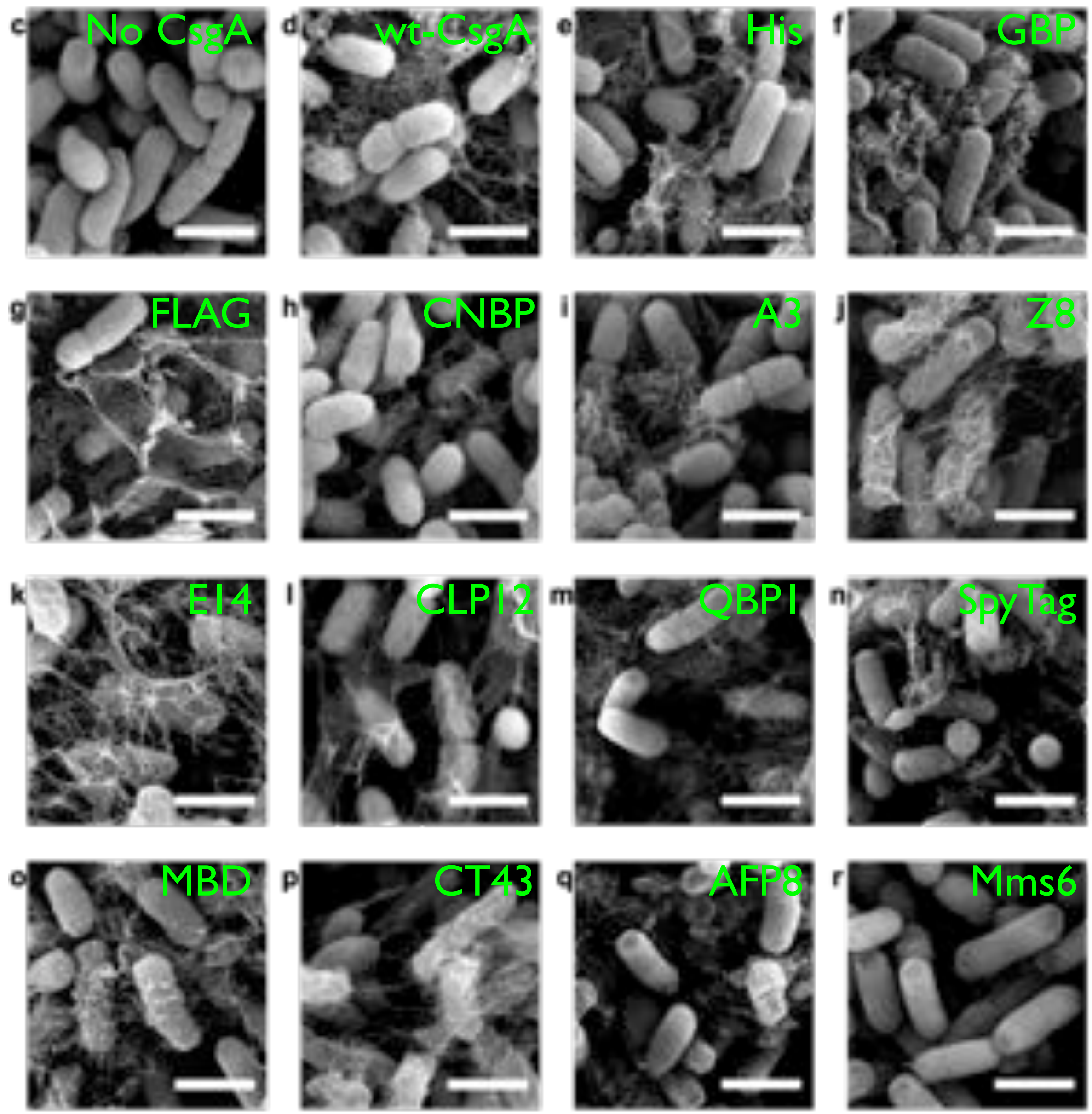
Quantifying CsgA-peptide production



→ Most peptides <50 amino acids do not hinder protein secretion or assembly

Peter Nguyen and Zsofia Botyanszki

Most CsgA-peptide mutants form amyloids



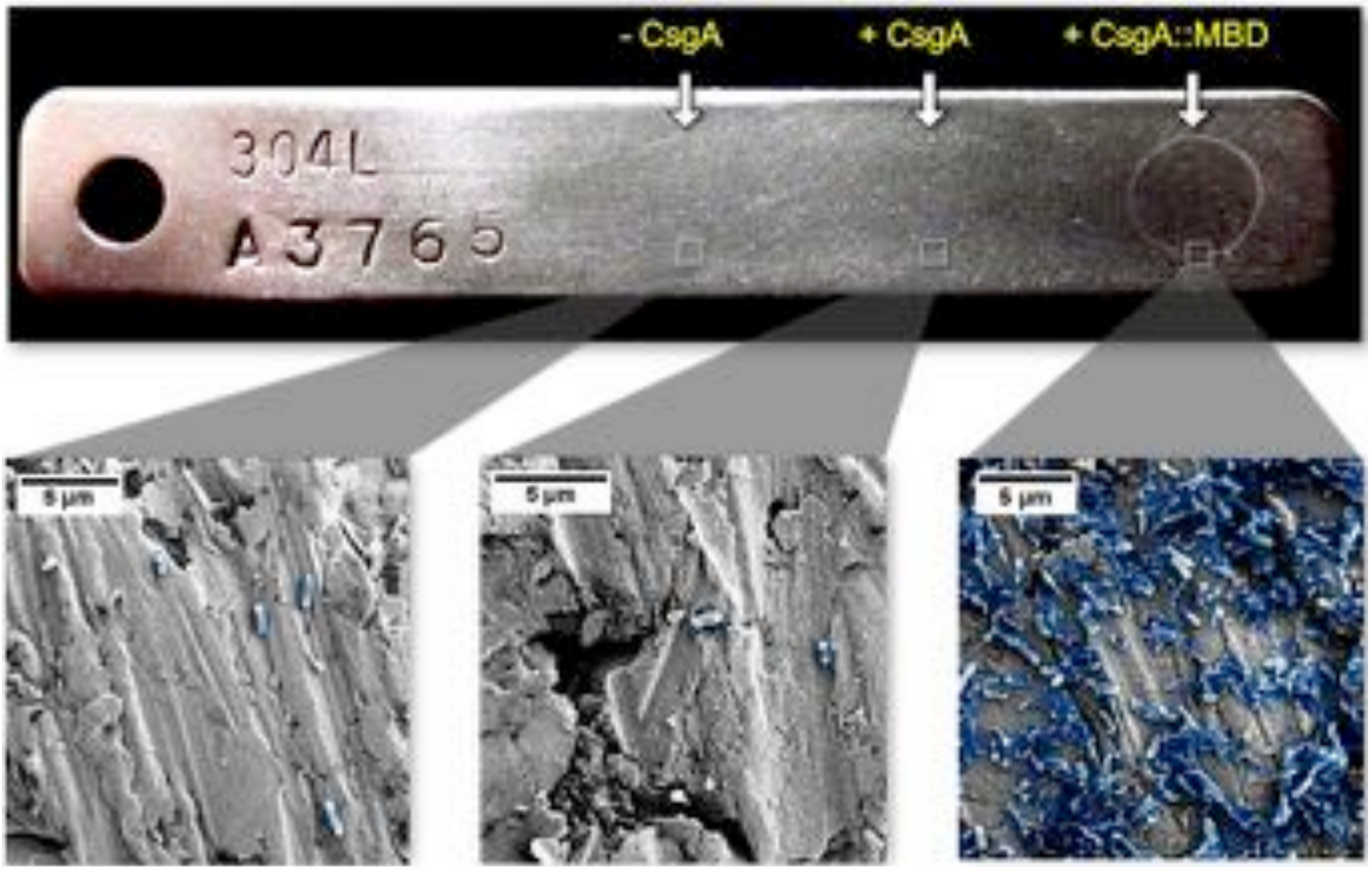
Scale bar = 1 µm

HARVARD School of Engineering and Applied Sciences
WYSS INSTITUTE

Peter Nguyen

CsgA-MBD enhances adhesion to steel

Growth of cells in suspension culture --> Induction of CsgA-MBD production --> Cultures spotted on coupon --> Vortexing wash --> Imaging by SEM



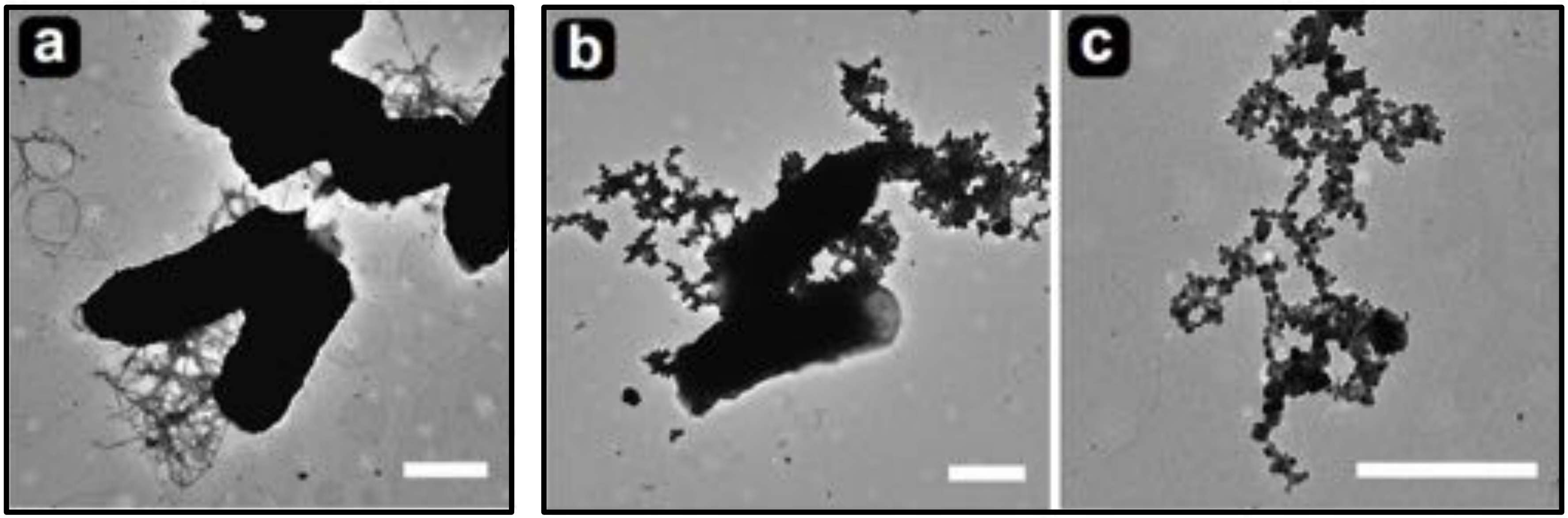
MBD	KCTSDQDEQFIPKGCSK	17	Substrate Binding	Binding to stainless steel surfaces	Mol. Microb. 2006, 59(4): 1083.

Harvard School of Engineering and Applied Sciences WYSS INSTITUTE

Peter Nguyen

CsgA-A3 templates AgNP growth

Grow of cells in suspension culture --> Induce CsgA-A3 production --> Form biofilm on TEM grid --> Incubate with $AgNO_3$ --> Stain and image



wt-CsgA

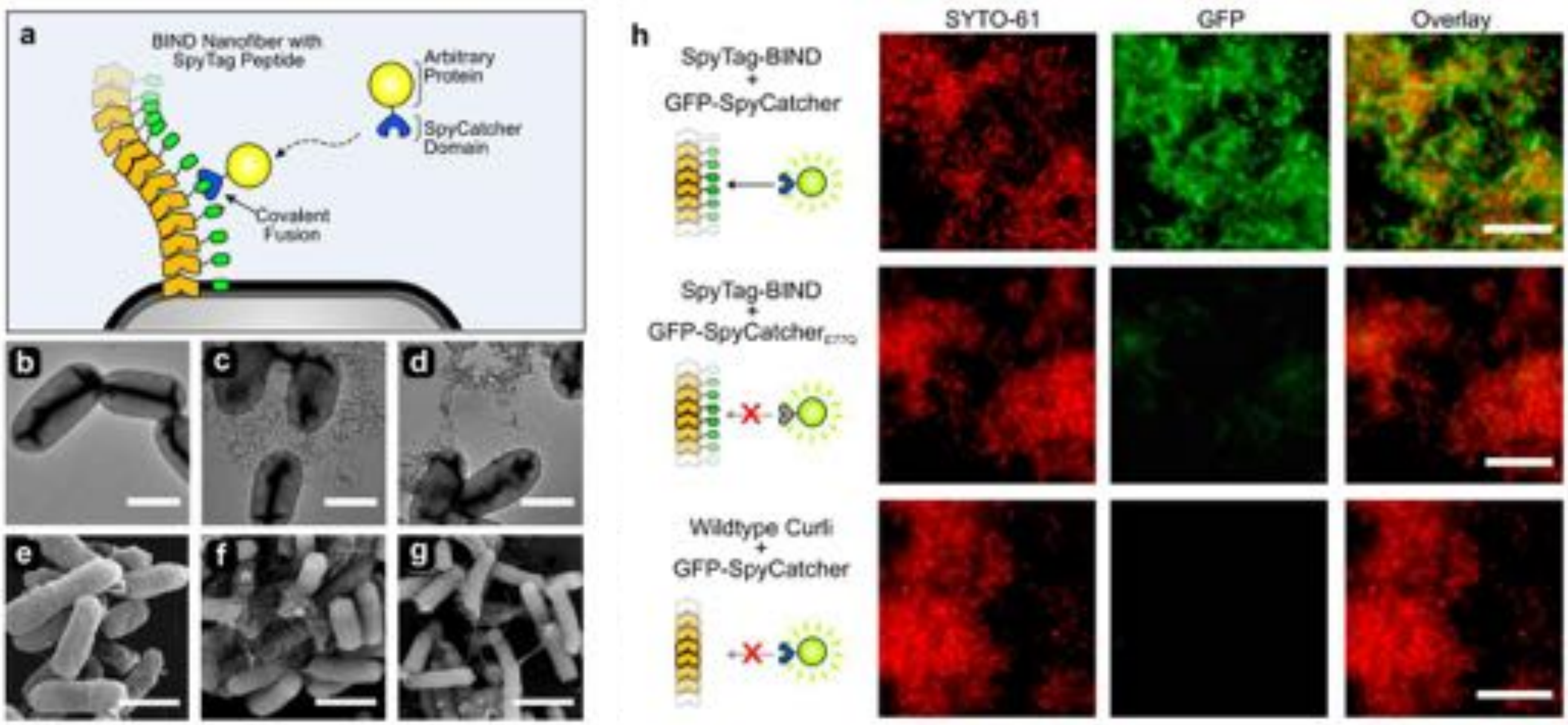
CsgA-A3

A3	AYSSGAPPMPPF	12	Substrate Binding	Gold surface binding	Small 2005, 1(11): 1048.

Peter Nguyen

CsgA-SpyT enable protein immobilization

Grow cells on glass substrate --> Induce CsgA-SpyT during growth --> Incubate with GFP-SpyC --> Wash un-bound protein --> Stain and image

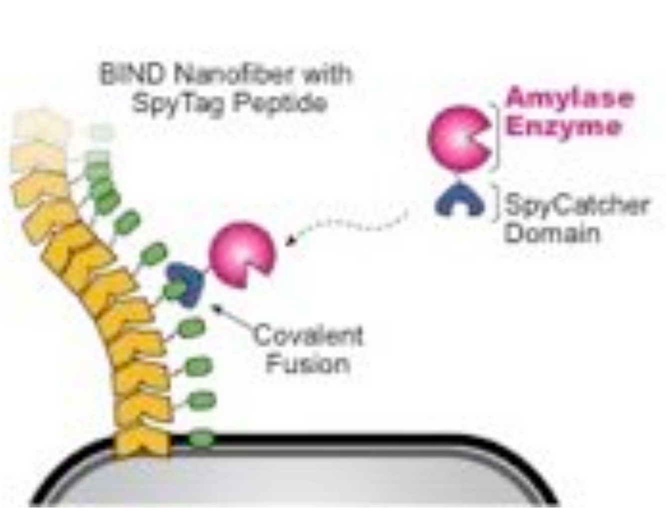


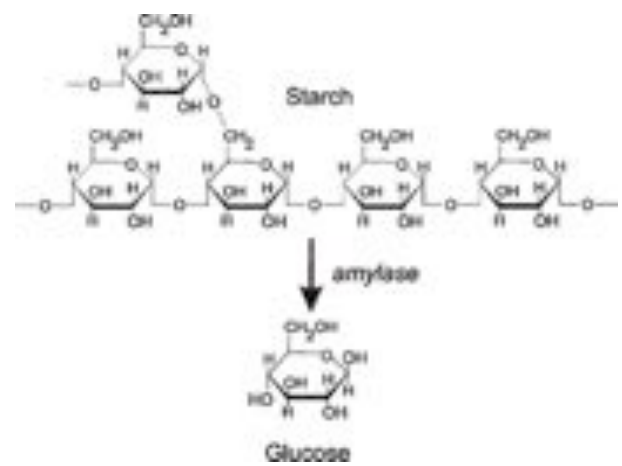
SpyTag	AHIVMVDAYKPTK	13	Protein Display	General covalent capture/display of proteins	PNAS 2012, 109(12): E690.

HARVARD School of Engineering and Applied Sciences   WYSS INSTITUTE

Zsofia Botyanszki

BIND as a catalytic substrate



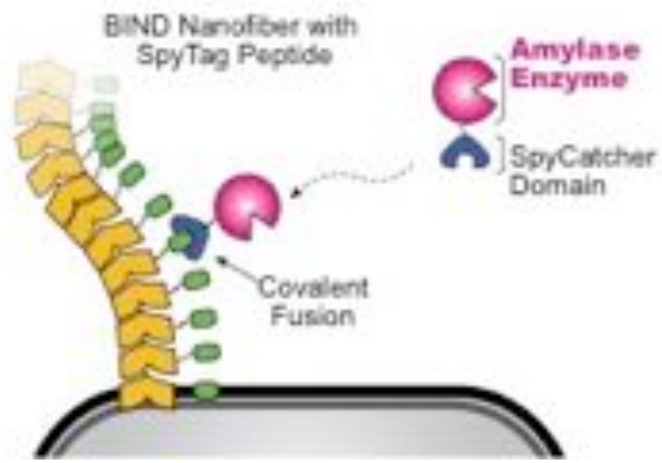


- Hydrolyzes alpha glycosidic bonds of sugars.
-
- Used for:
  - Ethanol production
  - HFCS production
  - Laundry detergents

- Accounts for ~***30%*** of world-wide industrial enzyme production

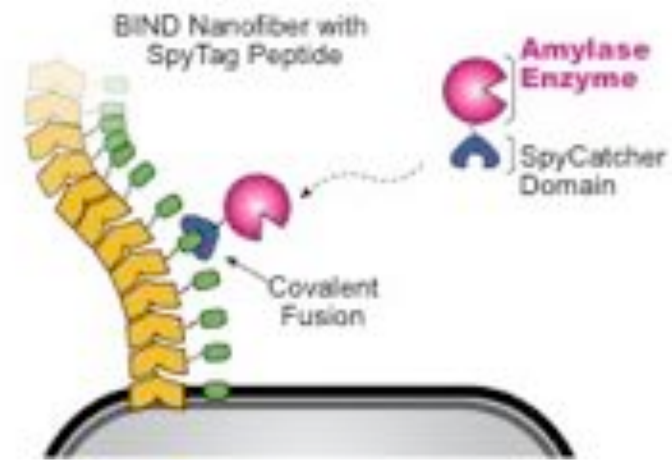
Zsofia Botyanszki

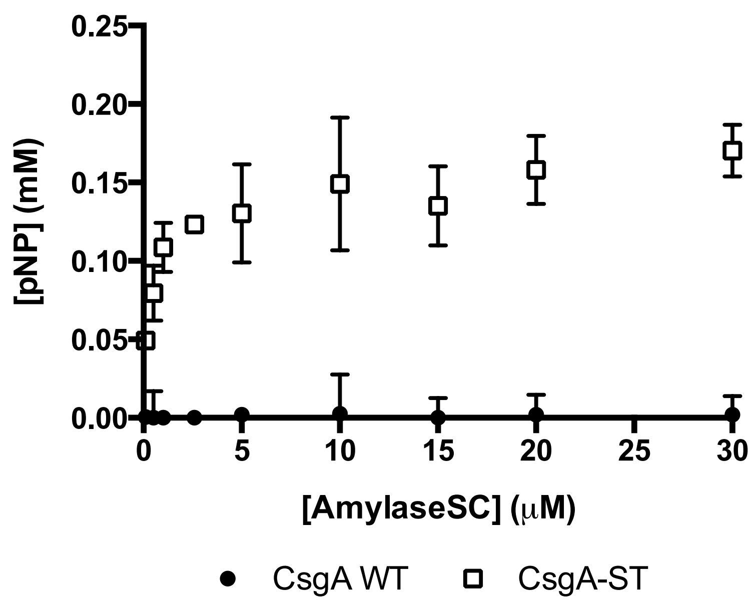
BIND as a catalytic substrate



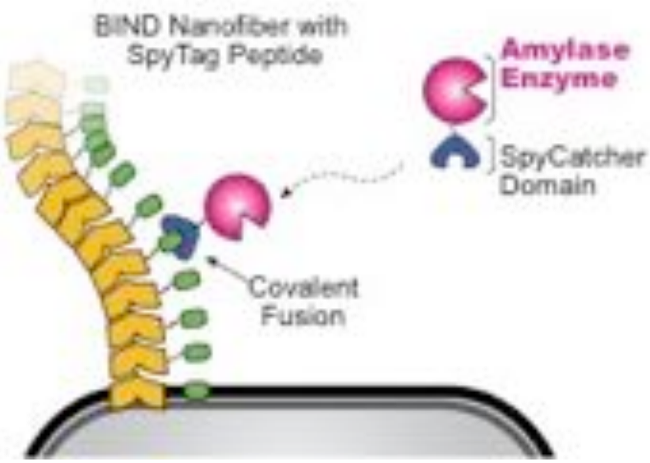
Grow cells in suspension culture --> Induce CsgA-SpyT in culture --> Filter through 0.2 µm filter --> Incubate with Amylase-SC --> Monitor catalytic activity

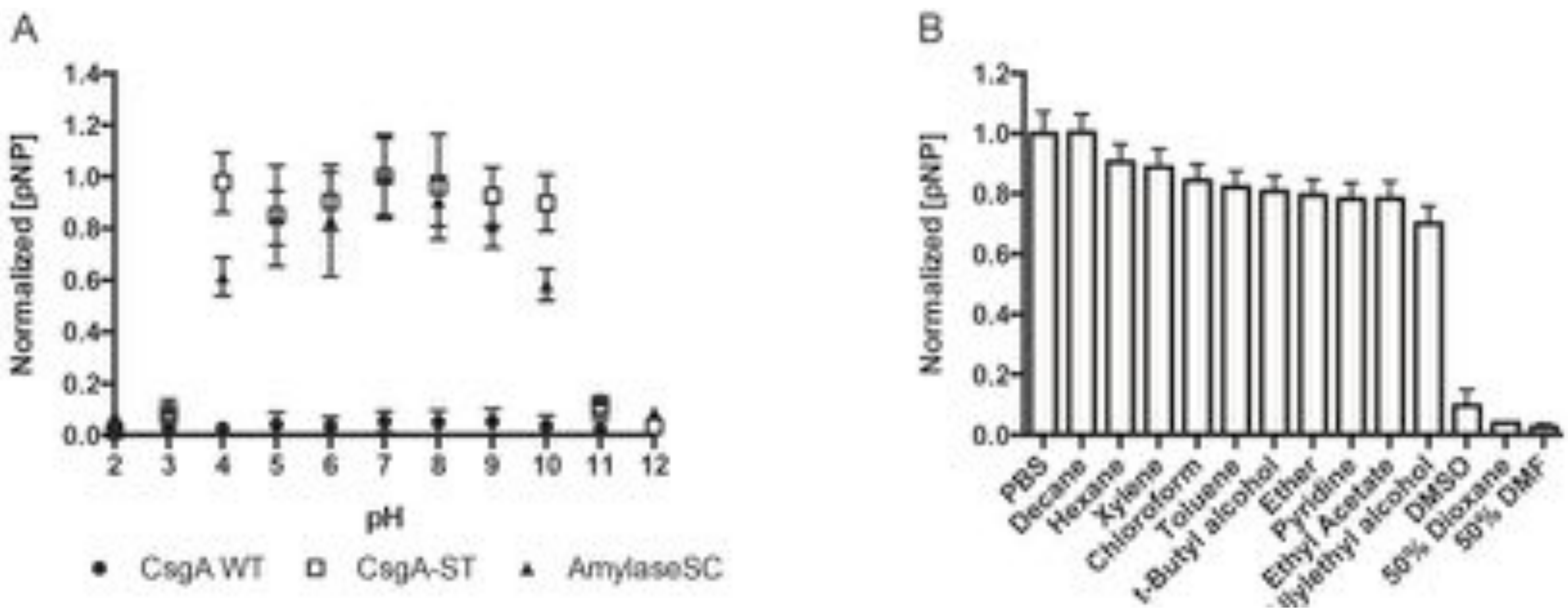
BIND as a catalytic substrate





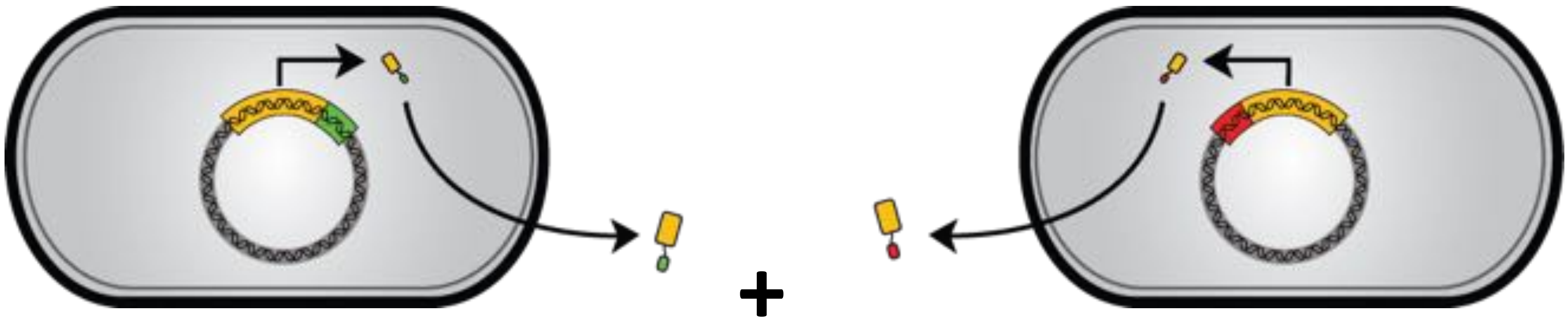
BIND as a catalytic substrate





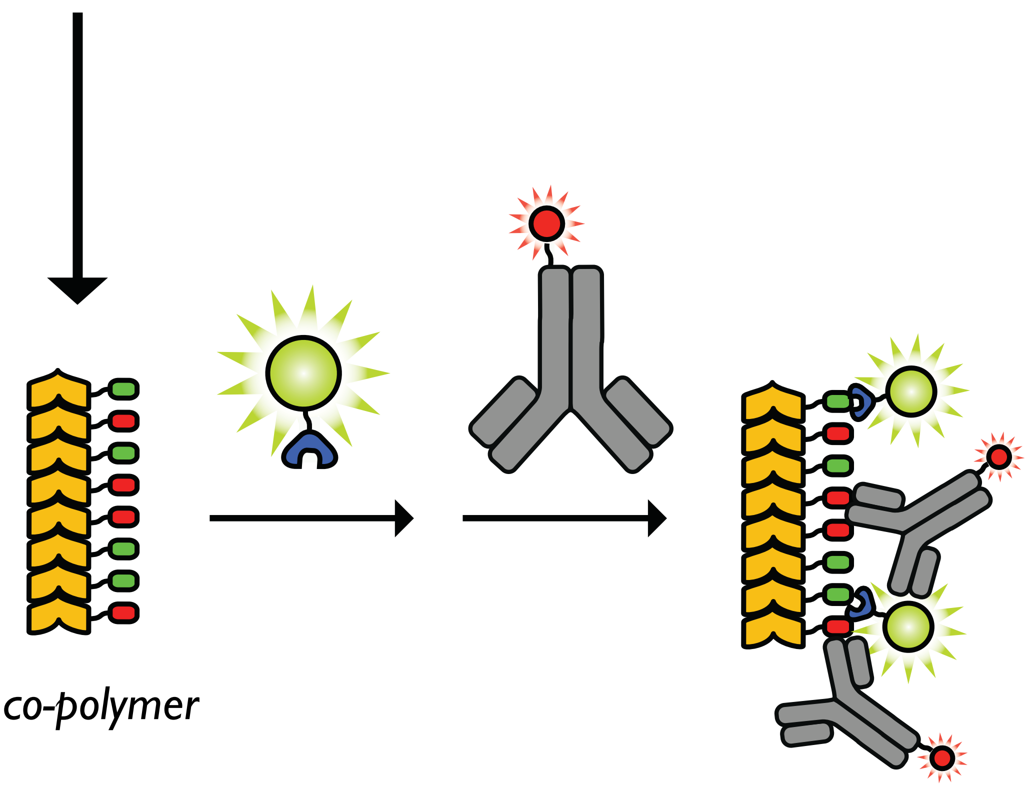
Zsofia Botyanszki

Co-assembly of Two CsgA Variants



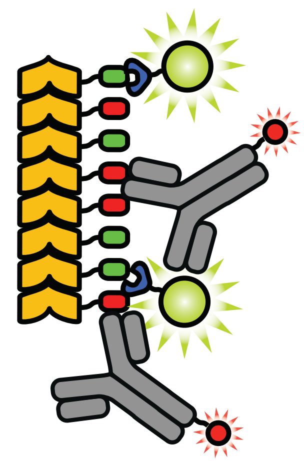
Combine BIND functions:

- Surface adhesion
- Catalysis
- Binding to soluble species



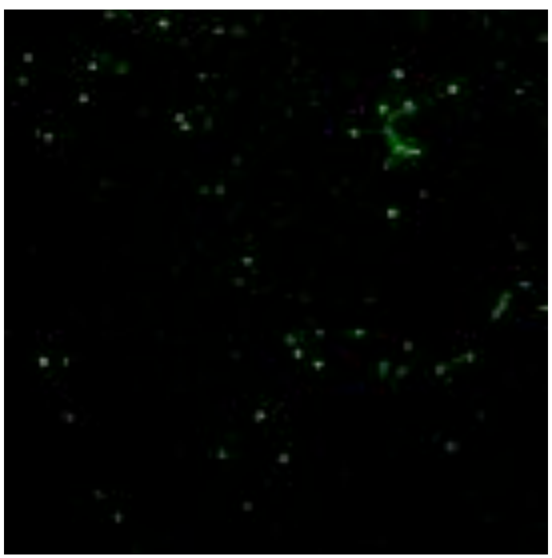
Bifunctional Curli Networks

Two CsgA::peptide variants can be displayed simultaneously to create multifunctional materials

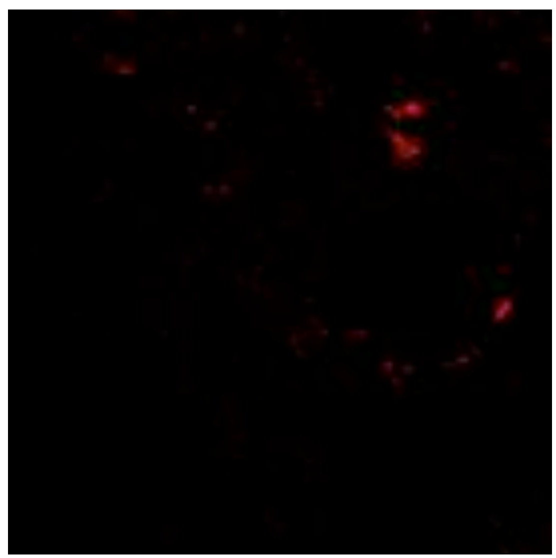


Function 1: SpyTag
(Venus::SC-immobilization)

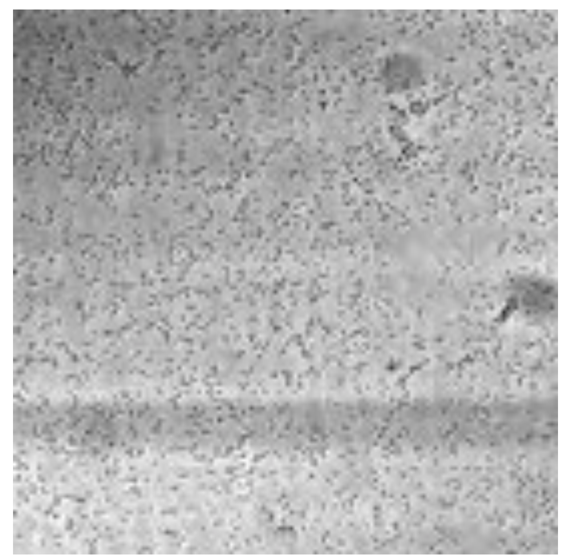
Function 2: FLAG tag
(anti-FLAG 1°, 633nm Dylight 2°)



GFP detection



DyLight 633 detection

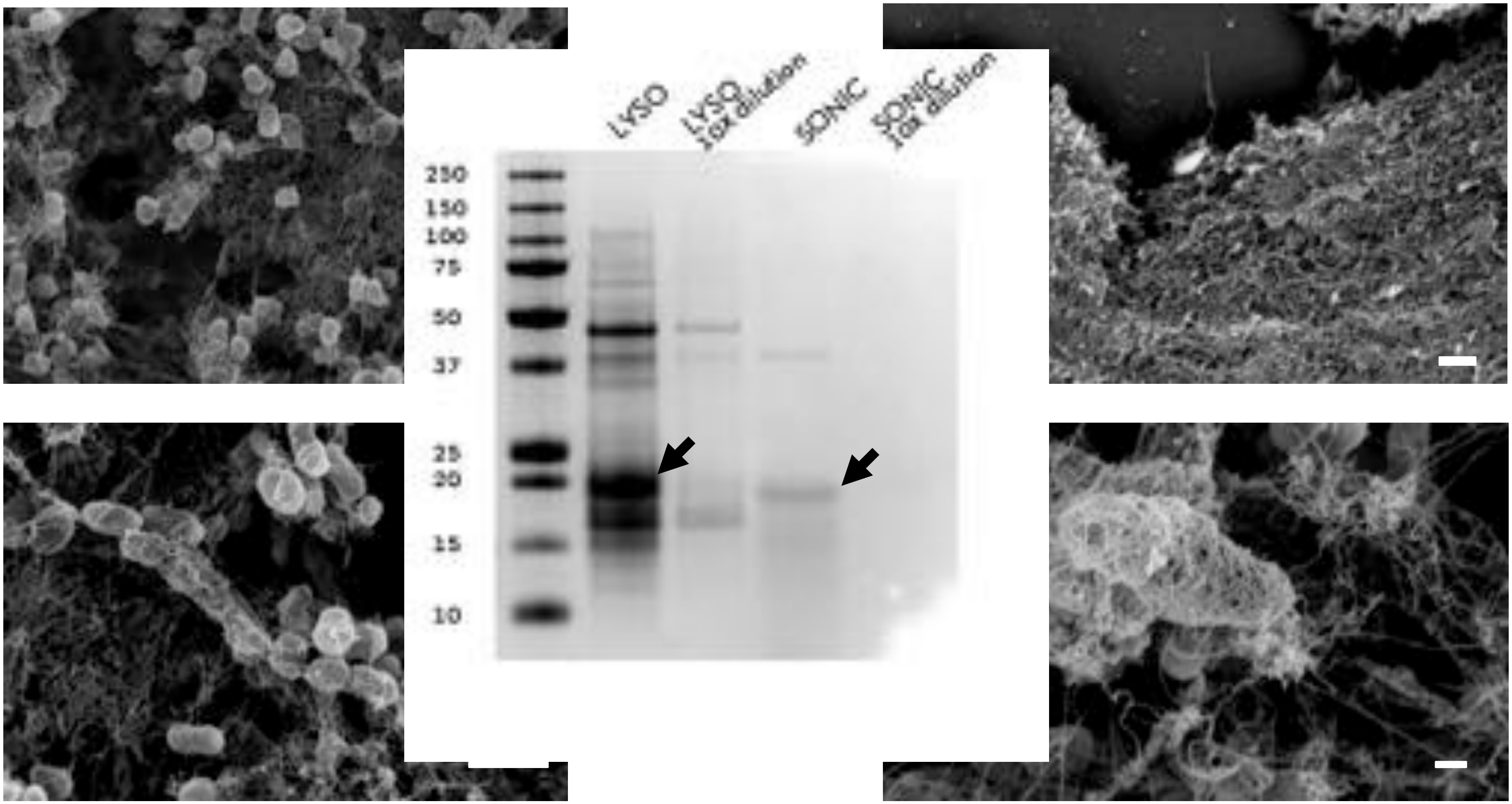


DIC

HARVARD School of Engineering and Applied Sciences
WYSS INSTITUTE

Peter Nguyen

Amyloid network survives decellularization



Scale bars = 1 µm

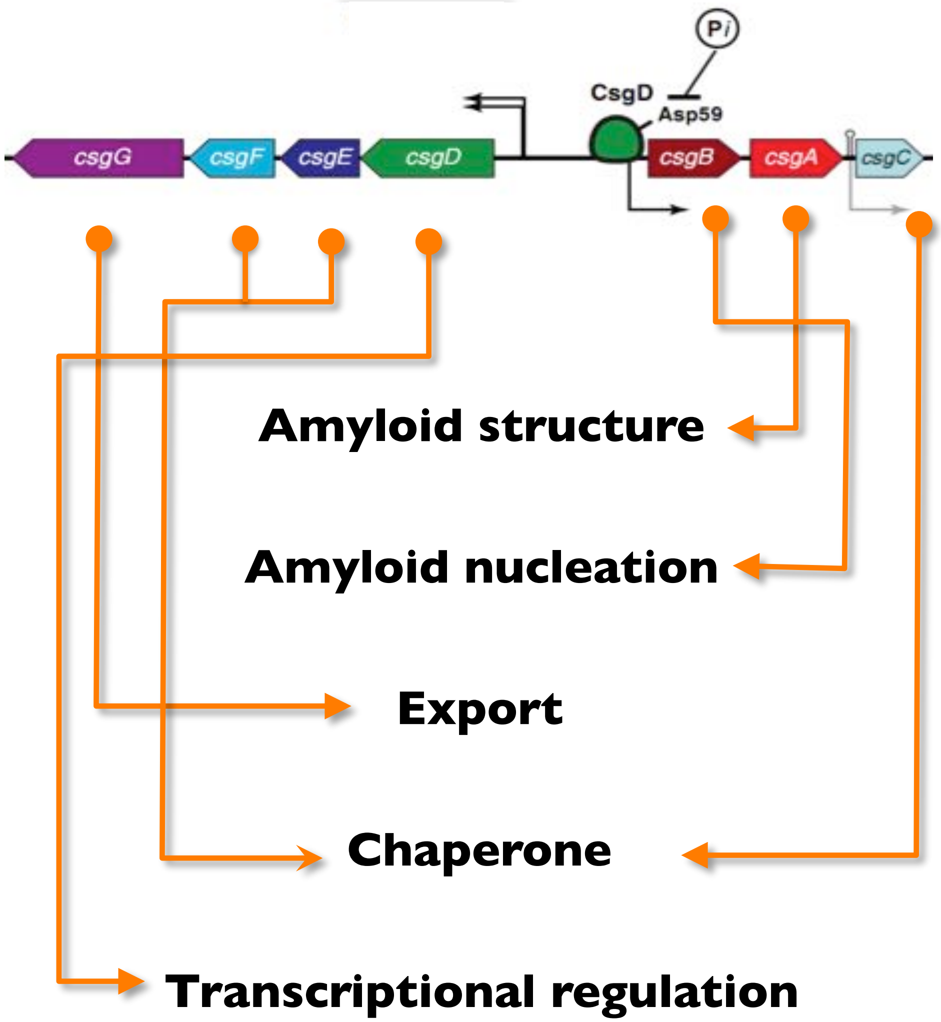
Scale bars = 200 nm

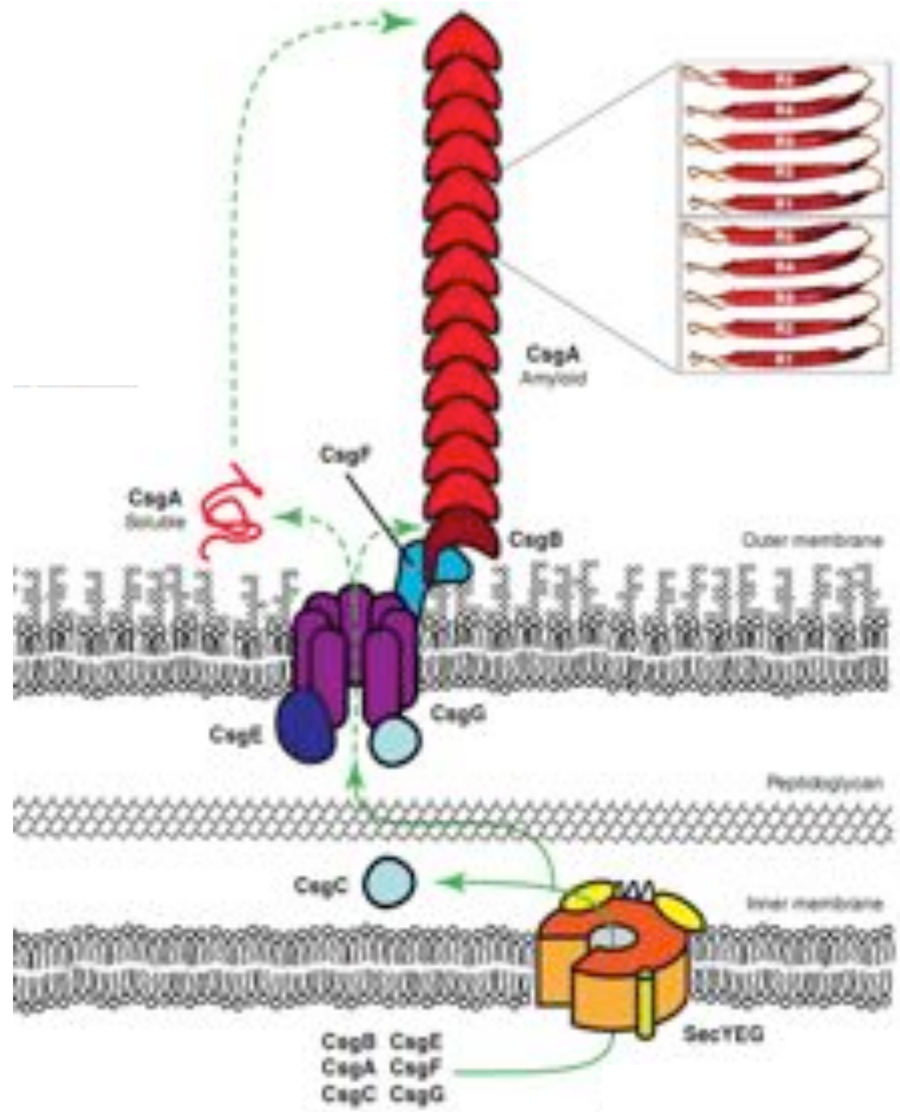
Amyloid material remains intact after harsh treatments (solvents, pH)

HARVARD School of Engineering and Applied Sciences
WYSS INSTITUTE

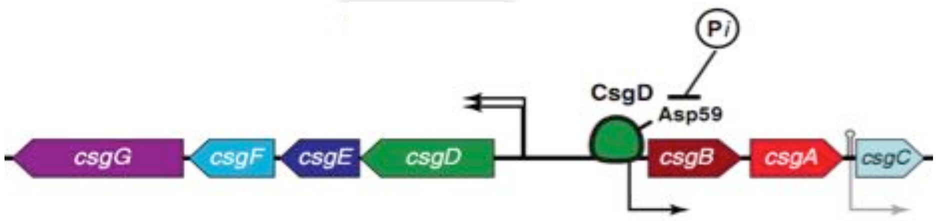
Peter Nguyen

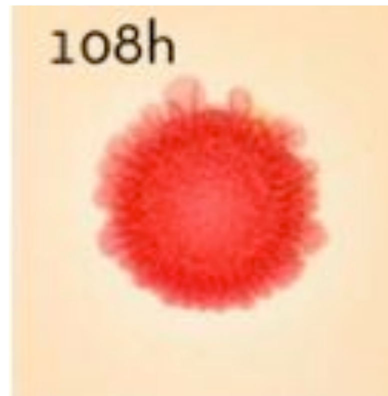
Curli operon optimization

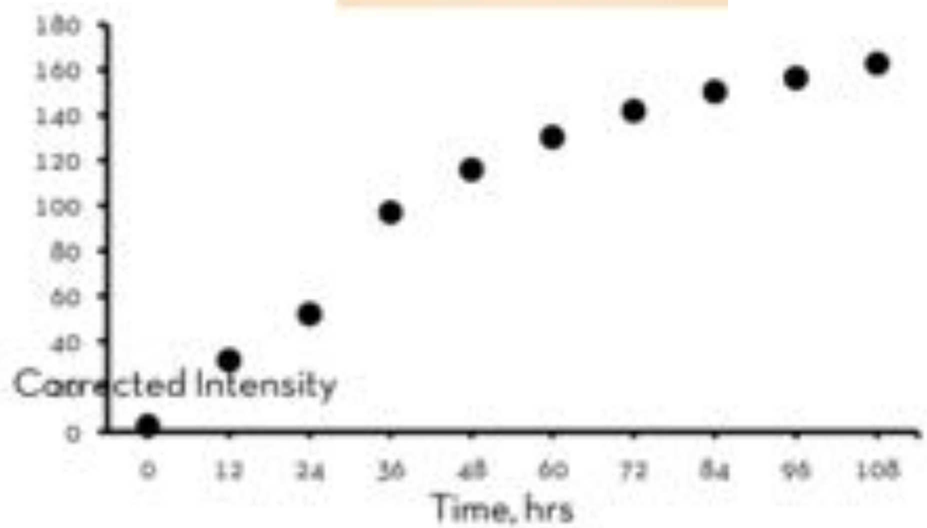




Curli operon optimization







Library of mutants with varying production levels for each music

↓

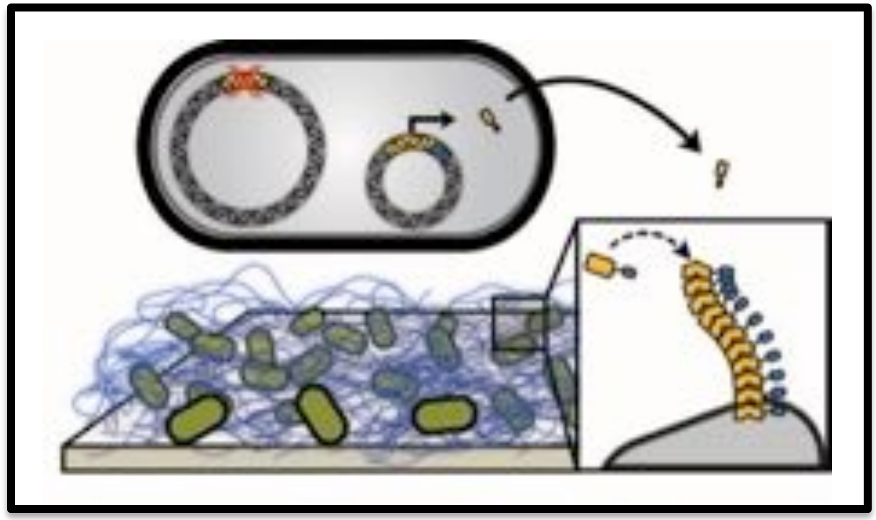
Screen library for amyloid formation

↓

Identify mutants with optimal curli production

BIND: what is it good for?

Combining the versatile functions of recombinant proteins with scalable materials fabrication

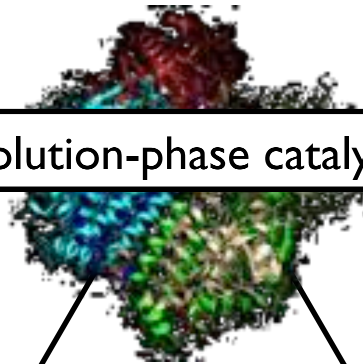


CAPABILITIES

- **Specific surface adhesion**
- **Nanoparticle templating**
- **Enzymatic catalysis**
- **Specific binding and capture of soluble entities**
  (metals, small molecules, proteins, viruses, cells)
- **Large scale material production**
  (biofilm paint, spray-on coatings, self-standing 3D materials)
- **Environmental responsiveness**
  (sensing, programmed formation/breakdown, dynamic properties)
- **Programmed biological interactions**
  (antimicrobial coatings, live biotherapeutic)

HARVARD School of Engineering and Applied Sciences   WYSS INSTITUTE

BIND for biocatalysis



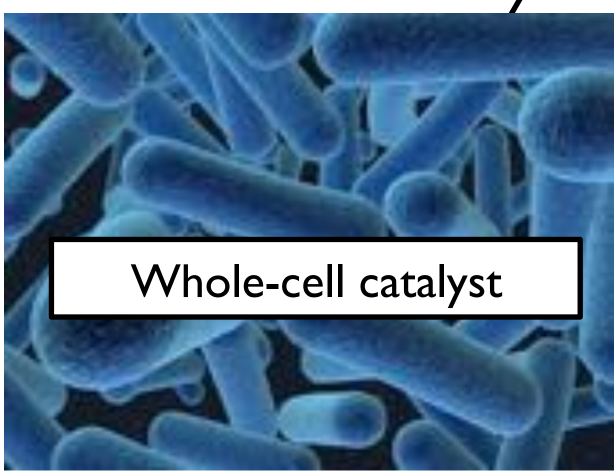
Solution-phase catalyst

- Poor catalyst recycling/ recovery
- Limited catalyst stability

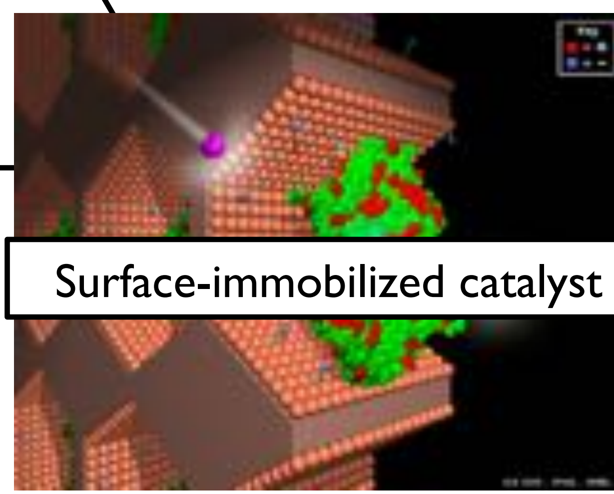
Existing biocatalysis strategies

- Limited substrate diversity
- Contaminants complicate product purification

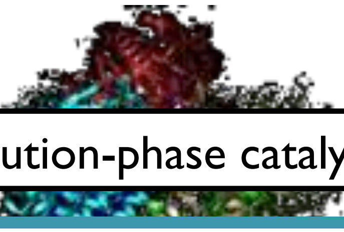
- Activity affected by immobilization
- Cost of substrate and processing



Whole-cell catalyst



Surface-immobilized catalyst



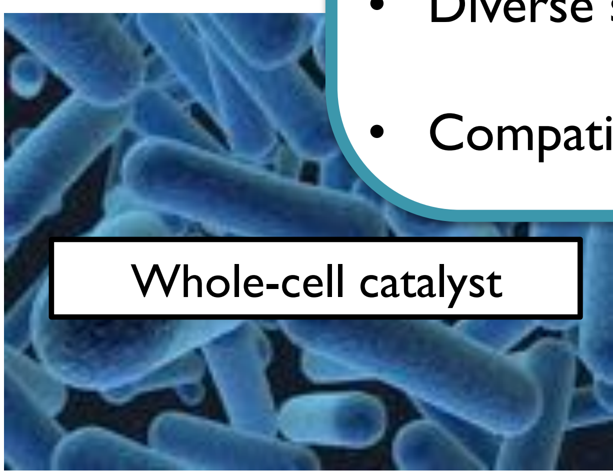
recycling/
tability

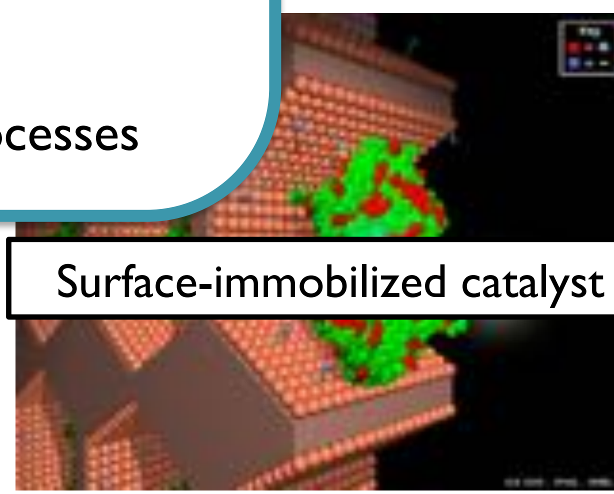
y affected by
ilization
f substrate and
sing

- Limited substr
- Contaminants
  product purifi

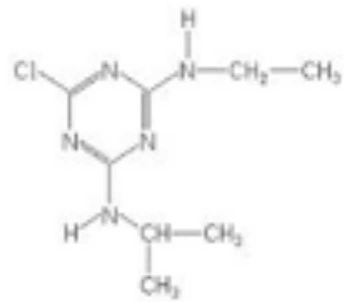
BIND for biocatalysis

- Modular immobilization strategy
- No enzyme purification or processing
- Enhanced enzyme stability
- Diverse substrate tolerance
- Compatible w/ continuous flow processes

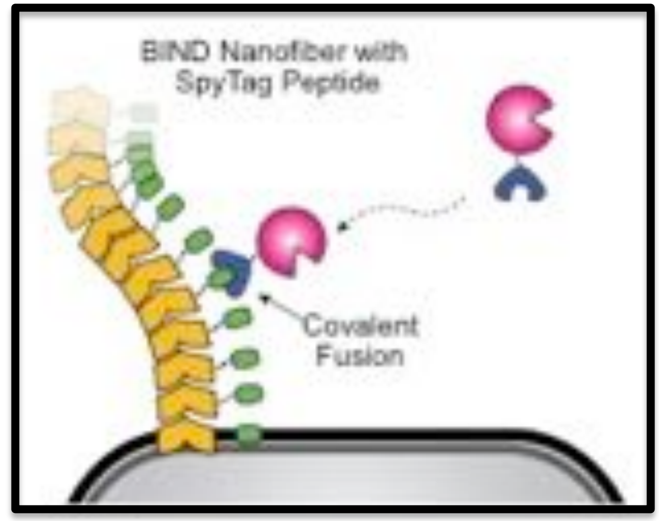




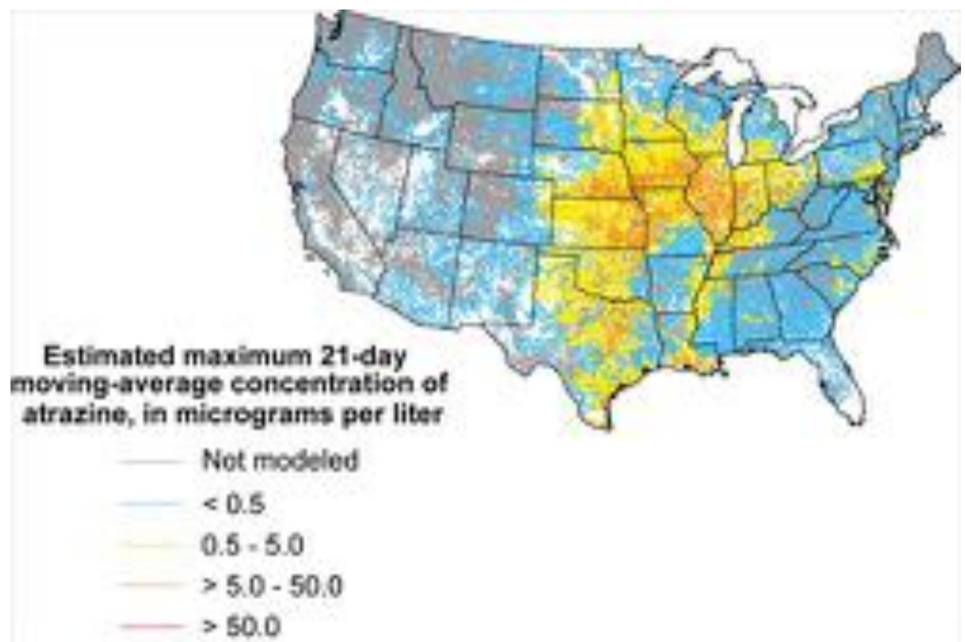
Enzymatic BIND for water decontamination



ATRAZINE

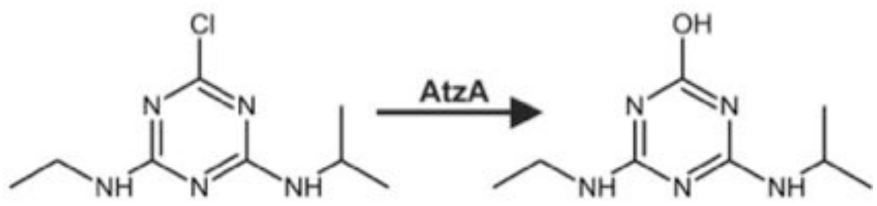


- EPA Maximum Contaminant Level (MCL) = 3ppb
- Frequently found to be above 5ppb in the mid-east and mid-west
- 0.1ppb = endocrine disruption

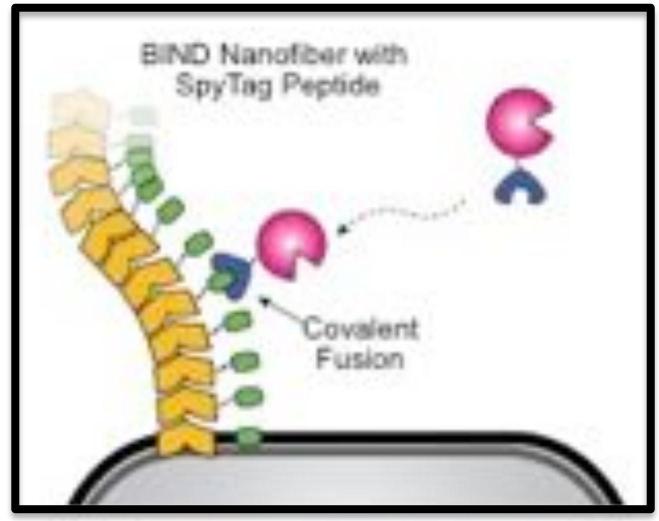


HARVARD School of Engineering and Applied Sciences
WYSS INSTITUTE

Enzymatic BIND for water decontamination



Atrazine 2-hydroxyatrazine



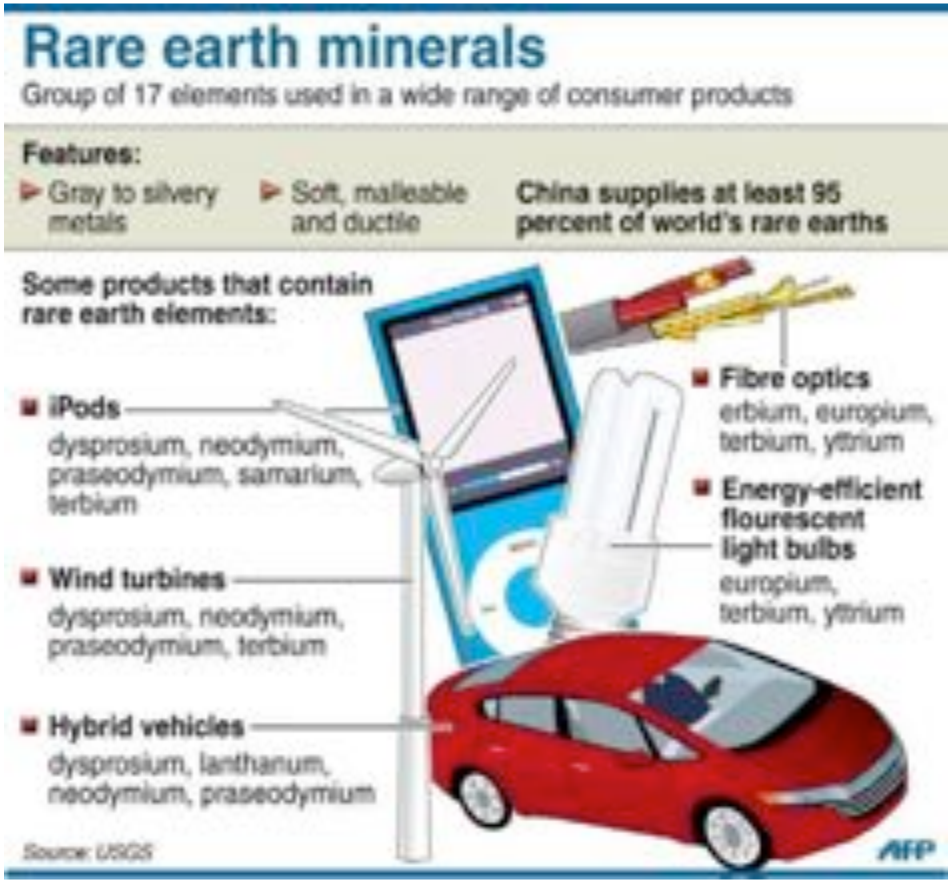
Enzymatic water decontamination is inefficient and expensive:

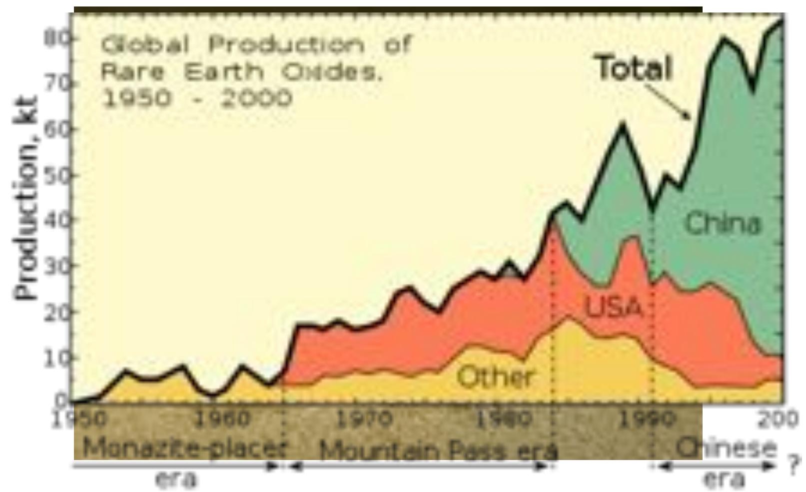
- Naturally occurring strains do not eliminate atrazine to acceptable levels
- Cost of enzyme purification is too high
- Limited substrate diffusion across cell membrane inhibits breakdown with whole cells

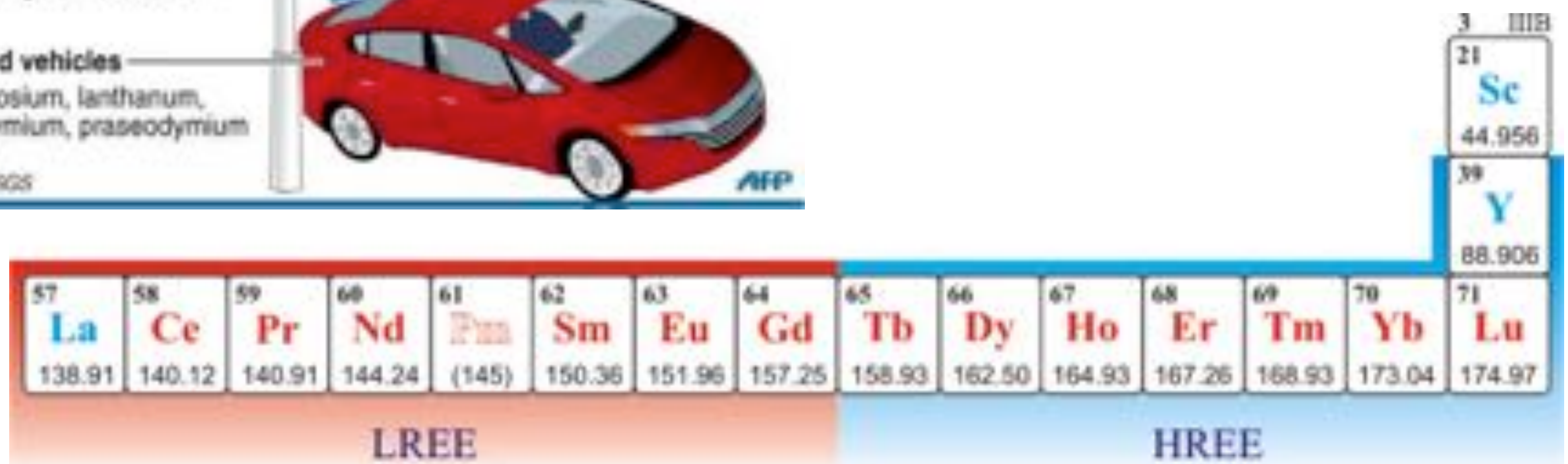
→ Can BIND facilitate an efficient continuous flow atrazine decontamination system by displaying enzymes?

BIND for specific metal removal/recovery

The need for rare earth metals

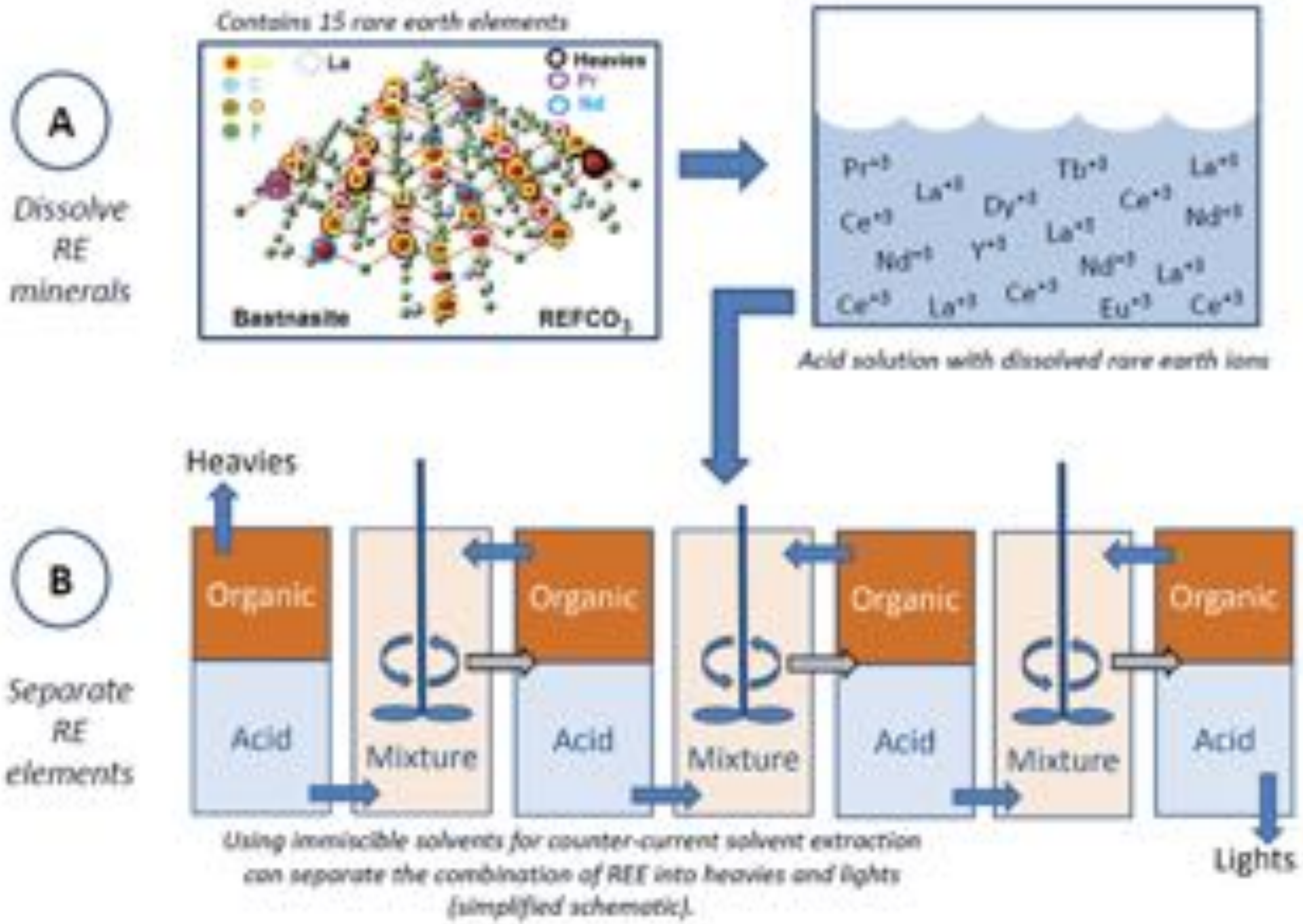






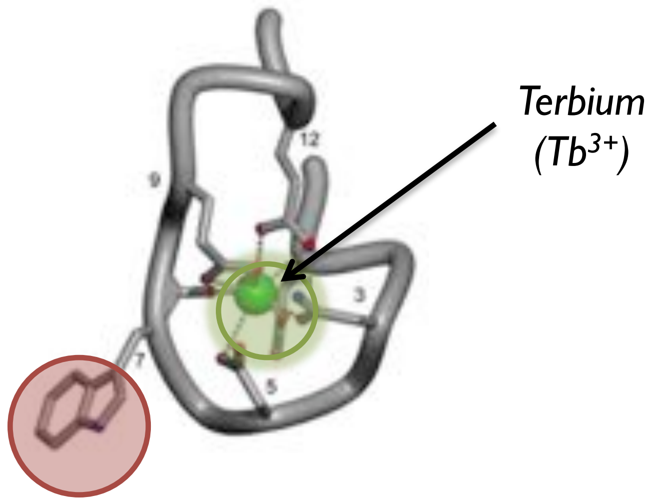
HARVARD School of Engineering and Applied Sciences
WYSS INSTITUTE

SEPARATING RARE EARTHS at MOUNTAIN PASS



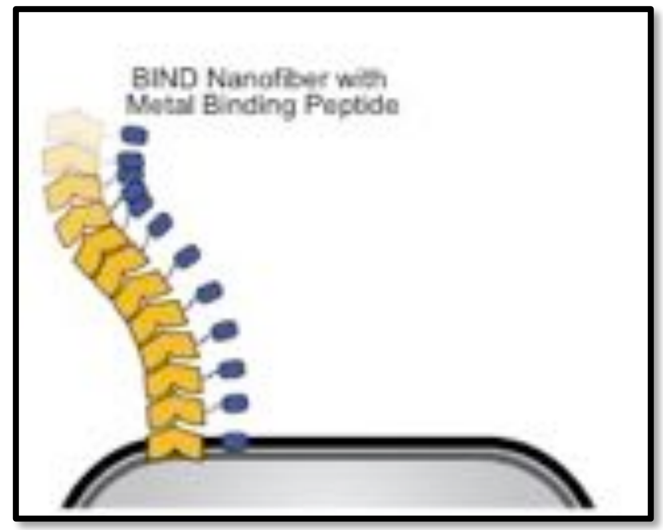
HARVARD School of Engineering and Applied Sciences WYSS INSTITUTE

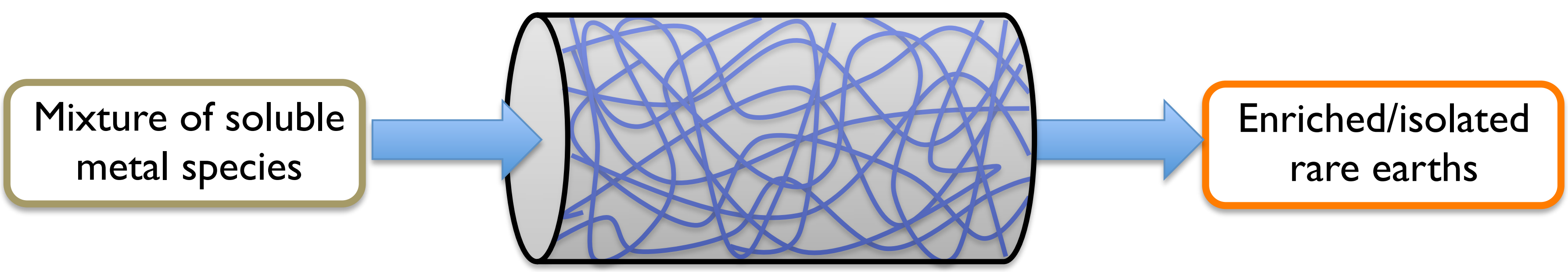
Rare earth isolation and separation



Lanthanide binding peptides

Nitz, et al. *Angewandte* **2004** *43*(*28*) 3682.





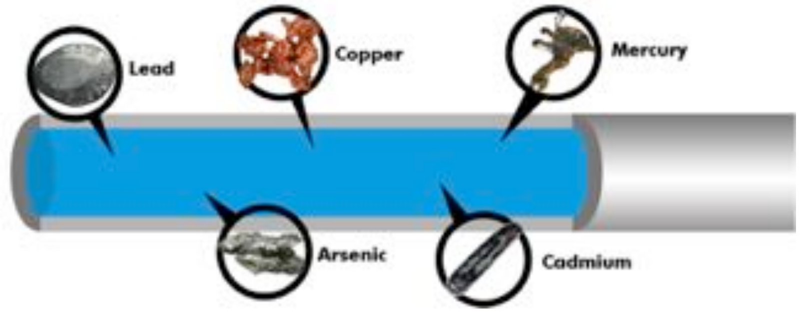
BIND matrix displaying rare earth binding domains

Richie Tay

Other possible BIND applications

BIOFUEL PRODUCTION

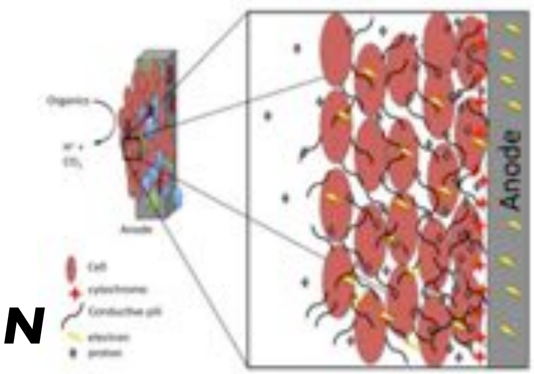
WATER PURIFICATION



CELL CAPTURE/ AFFINITY SEPARATIONS

PROTECTIVE COATINGS

BIO-ELECTRODE INTERFACE



BIOSENSING DECONTAMINATION

Benefits of Biofilm Technology

- Self-generated and self-renewing scaffold; the bacterium as a nanomaterial factory.
- Vast surface area for immobilization.
- Robust – stable under conditions normally considered harsh for biology

- Easily scalable – could lead to cost effective large scale solutions.
- Living material – may allow for dynamic temporal control over material properties
- A green technology for nanomaterials.

Acknowledgements

Joshi Group
Glenna Meister
Peter Nguyen
Dan Rubin
Zsofia Botyanszki
Rajiv Desai
Richie Tay
Pichet Praveschotinunt
Anna Duraj-Thatte

Collaborators
Christine Ortiz (MIT)
Michael Gevelber (BU)
Ali Miserez (NTU, Singapore)

Wyss Institute

Harvard Milton Fund

mrsec @harvard
materials science at the interface